JOURNAL OF AGRICULTURAL RESEARCH

VOL. 43 WASHINGTON, D. C., DECEMBER 1, 1931 NO. 11

HARDINESS STUDIES WITH 2-YEAR-OLD ALFALFA PLANTS [1]

By GEORGE L. PELTIER, *Plant Pathologist, Nebraska Agricultural Experiment Station,* and H. M. TYSDAL, *Associate Agronomist, Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Because of an apparent relationship between winter injury and bacterial wilt (*Aplanobacter insidiosum* McC.), an investigation of some of the pathological and physiological aspects of alfalfa failures in Nebraska was undertaken in 1927. As a phase of this investigation an extensive study of winter injury under controlled conditions was begun. It is the purpose of this paper to present the results obtained with 2-year-old field-grown plants of nine different kinds of alfalfa (*Medicago sativa*), exposed to a temperature of approximately −18° C. for various lengths of time, on nine different dates during the season of 1928–29. In all, more than 15,000 plants were used in these tests, a number thought to be sufficiently large to yield consistent results. No attempt will be made to review the voluminous literature on winter injury of alfalfa or other crop plants, and only such mention will be made of the literature as has a direct bearing on the subject herein discussed.

METHODS AND PRELIMINARY STUDIES

KINDS OF ALFALFA

Seedings were made April 5, 1927, in plots 7 feet wide and 100 feet long. The following alfalfas were used in the tests—Hardy: Grimm (commercial No. 632), Ladak (F. C. I.[2] No. 14135), Turkestan (commercial No. 2674); mid hardy: South Dakota No. 12, Nebraska common, Utah common; nonhardy: Arizona common, Italian (F. C. I. No. 2123), and South African (F. P. I. No. 66729).

RELATION OF FIELD STANDS TO DIAMETER AND BRANCHING OF ROOTS

Uniform stands were obtained with all alfalfas during the first season. At the end of the first winter some thinning out occurred in the more nonhardy alfalfas, and after the second winter stand reduction was still more pronounced in the same plots. In order to ascertain the number of plants per square foot plant counts were

[1] Received for publication May 26, 1931; issued January, 1932. Paper No. 106 of the Journal Series of the Nebraska Agricultural Experiment Station. This paper, the first of a series on hardiness in alfalfa, is based on cooperative investigations between the Department of Plant Pathology, Nebraska Agricultural Experiment Station, and the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] The letters F. C. I. and F. P. I. indicate, respectively, the accession numbers of the Division of Forage Crops and Diseases and the Division of Foreign Plant Introduction, Bureau of Plant Industry, U. S. Department of Agriculture.

made of all nine alfalfas in the spring of 1929. As the plants were dug, the root diameter just below the crown was measured with a wedge-shaped caliper and a record was made of the plants with single or branched roots. Notes on between 600 and 800 plants of each of the nine alfalfas were taken. In Table 1 are listed the number of plants found per square foot, the average root diameter, and the percentage of plants with branched roots. It will be noted

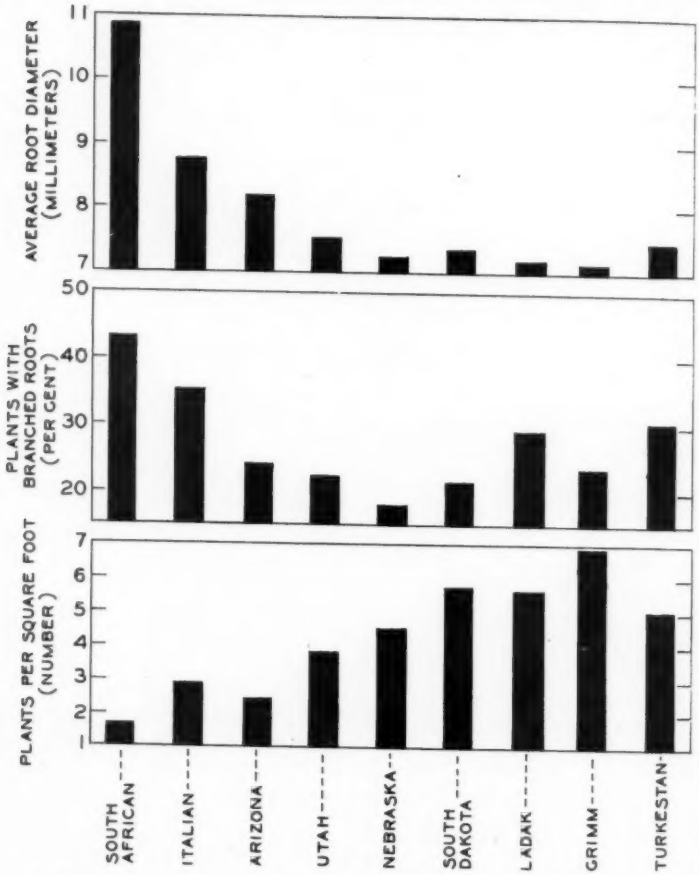

FIGURE 1.—Relation of field stands to root diameter and branching tendency of the nine alfalfas used in the artificial freezing tests

that there were fewer plants per square foot in the nonhardy alfalfas than in the mid hardy or hardy alfalfas.

Judging from the data in Table 1 and Figure 1, there is a correlation of -0.6887 ± 0.1182 between the number of plants per square foot and the average diameter of the roots. There does not appear to be a decided tendency for one alfalfa to produce more branched roots than others, since the difference in branching can be explained largely by differences in stand. The significance of root diameters is brought out in the artificial freezing tests.

TABLE 1.—*Field stand counts made in March, 1929, of the nine alfalfas used in the artificial freezing tests, together with the average root diameter and percentage of plants with branched root systems*

Kind of alfalfa	Plants per square foot [a]	Plants with branched roots	Average diameter of roots
Hardy:	*Number*	*Per cent*	*Millimeters*
Turkestan	5.0	30	7.46
Grimm	7.0	23	7.10
Ladak	5.6	29	7.14
Average	5.9	27	7.23
Mid Hardy:			
South Dakota	5.7	21	7.38
Nebraska	4.5	18	7.20
Utah	3.9	22	7.56
Average	4.7	20	7.38
Nonhardy:			
Arizona	2.4	24	8.18
Italian	2.9	35	8.72
South African	1.6	43	10.90
Average	2.3	34	9.27

[a] Uniform and comparable stands were present in all alfalfas in 1927.

TABLE 2.—*Moisture content of alfalfa plants at nine dates during the season of 1928–29*

Date of sampling	Average percentage of moisture in—			
	Three hardy alfalfas	Three mid-hardy alfalfas	Three nonhardy alfalfas	All
Oct. 15	72.0	69.6	70.3	70.6
Oct. 29	65.3	63.9	65.7	65.0
Nov. 13	64.3	63.7	64.2	64.1
Nov. 26	63.4	65.4	63.8	64.2
Dec. 18	64.6	64.6	63.6	64.3
Average	65.9	65.4	65.5	65.6
Mar. 12	62.2	63.4	62.4	62.7
Mar. 26	63.9	62.8	61.1	62.6
Apr. 9	67.4	65.0	60.1	64.2
Apr. 30	69.6	67.7	60.2	65.8
Average	65.8	64.7	61.0	63.8
Grand average	65.9	65.1	63.5	64.8

MOISTURE CONTENT AND GROWTH OF PLANTS

The percentage of moisture in the plants was determined in duplicate for each alfalfa on the various dates of freezing. (Table 2.) Representative plants prepared for the freezing tests, as described later, were used for these determinations. Table 2 shows that the percentage of moisture in all alfalfas tended to drop on each subsequent date until March. As the season advanced, the moisture content again increased in the hardy and mid hardy but remained low in the nonhardy alfalfas.

Observations on the general growth conditions of the field plants made March 26, 1929, indicated that the hardy, mid hardy, and nonhardy groups were good, fair, and poor, respectively, while actual

measurements of the new growth averaged 3.5, 3, and less than 1 cm. for the same three groups. Since this was the initial growth of the spring, it was interesting to note that following the comparatively severe winter the hardy alfalfas had a more vigorous growth earlier in the season than the nonhardy.

PREPARATION OF PLANTS FOR ARTIFICIAL FREEZING TESTS

The plants were dug so that 6 or more inches of the roots were obtained. Immediately after the plants were dug they were wrapped in burlap bags, placed in cold storage, and kept there until they were withdrawn for freezing. In no instance, however, did any of these plants stay in cold storage longer than 24 hours. The dirt was cleaned from the roots; the top growth, if any, was cut to 2 or 3 inches above the crown and the roots 6 inches below. Each plant was individually labeled and the following notes were taken: Diameter of the root approximately 1 cm. below the crown, number of stems and buds, together with the general condition of the plant, and the amount and

FIGURE 2.—Felt containers in which the alfalfa plants were exposed to artificial freezing

types of rots generally found in alfalfa plants, including any visible signs of winter injury that had previously occurred in the field.

After the notes were taken the plants were placed in felt containers (fig. 2) in which the roots were somewhat protected from the cold and only the crowns were exposed directly to the low temperatures. This method was followed because it was found by the senior author in freezing experiments the previous year that when the entire roots were exposed to low temperatures, considerable injury was caused to the lower ends, even though the crowns remained uninjured. This condition prevented normal development of the new rootlets when they were transplanted in the greenhouse. By the use of the felt containers, no noticeable injury occurred on the lower ends of the roots during the freezing tests.

Ninety plants of each alfalfa were prepared for each freezing test, as well as 20 which were not frozen and served as controls. Thirty plants of each alfalfa were withdrawn after an exposure in the freezing chamber of ½, 1, and 2 hours, respectively.

TEMPERATURES IN THE FREEZING CHAMBER

All plants were exposed to a uniform temperature of approximately −18° C. The control freezing equipment employed in these experiments has recently been described by the senior author (*3*).[3] Although

[3] Reference is made by number (italic) to Literature Cited, p. 955.

the temperature of the freezing chamber was −18° or below, it usually went up a few degrees when the plants were placed in the chamber. The first reading was taken directly after the plants were placed in the chamber, which accounts for the higher temperatures recorded. After about 10 minutes the temperature was usually again about −18°. Temperatures were usually taken at 10-minute intervals while the plants were exposed in the freezing chamber. On the whole, an average temperature of near −18° was maintained throughout the course of the tests, the range varying from 2 to 5 degrees. Temperatures were recorded for the most part by thermocouples.

While the plants were exposed to a temperature of approximately −18° C. for various lengths of time, the actual temperature near the plants was not known. By the use of thermocouples in direct contact with the roots at the bottom of the felts and the crowns exposed above the felts, the exterior temperature of the plants was measured. The average of five such determinations is given in Table 3.

TABLE 3.—*Actual temperatures, recorded by means of thermocouples, of the alfalfa plants exposed in the freezing chamber held at a temperature of −18° C.*

[Average of five determinations]

Time	Temperatures		
	At the root	At the crown	Above the plants
	°C.	°C.	°C.
0.5 hour	0.62	−9.10	−18.40
1.0 hours	−4.52	−10.82	−19.20
2.0 hours	−9.70	−14.94	−18.47

TREATMENT OF PLANTS AFTER EXPOSURE TO LOW TEMPERATURES

As soon as the plants were frozen, they were well wrapped in burlap bags and placed in a cold-storage room until they could be planted. Within six hours after freezing, the exposed plants, together with the nonfrozen controls, were planted in a bed in the greenhouse. That this was the best procedure was determined in a preliminary experiment on the influence of the rate of thawing on the ultimate survival of 400 plants of Grimm alfalfa exposed to a temperature of −18° C. for 1½ hours. The percentage of survival recorded one month after freezing indicated that the rapidity of thawing influences the total number of plants that survive. Of the plants thawed out rapidly under running water (18°), only 36 per cent survived; of those wrapped in burlap bags and left at room temperature (18°) for 1½ hours, 62 per cent survived; of those wrapped in burlap bags and held in a cold-storage room (5°) for 1½ hours, 84 per cent survived; while of those buried in moist sand (0°) and gradually warmed for 20 hours, 96 per cent survived. These results show the desirability of a uniform treatment of the plants after freezing in comparative tests.

Notes on the recovery of the plants were taken at intervals of 2, 4, and 8 weeks, at which time the plants were dug and detailed records taken, including the number of stems and height of the new growth, the amount of crown and phloem injury, the extent of internal discoloration caused by freezing, and the types of cold injury present.

Since a history of each plant was obtained prior to freezing and again at the end of the experiment, a check on the behavior of the individual plants to exposure to low temperatures could be ascertained.

ANALYSIS OF DATA

For analyzing the data a card containing all the records for each plant was made up, and by means of punching, sorting, and tabulating machines the data were analyzed for any one or any combination of factors desired. The use of this rapid and accurate method greatly facilitated analysis.[4]

EXPERIMENTAL RESULTS

EFFECT ON FIELD-GROWN PLANTS PRODUCED BY EXPOSURE TO A TEMPERATURE OF −18° C. FOR ONE HOUR

As previously stated, 2-year-old field plants of 9 alfalfas were exposed to a controlled temperature of about −18° C. for 3 periods—½, 1, and 2 hours—on 9 different dates during the fall of 1928 and the spring of 1929. In Table 4 the percentage of survival of the controls and the plants exposed for 1 hour on the 9 dates are presented.

TABLE 4.—*Percentage of alfalfa plants of the nine sorts that survived an exposure of one hour to a temperature of −18° C. at nine periods during the season of 1928–29*

Kind of alfalfa	Percentage survival [a]																	
	Oct. 15		Oct. 29		Nov. 13		Nov. 26		Dec. 18		Mar. 12		Mar. 26		Apr. 9		Apr. 30	
	Controls	Frozen	Controls	Frozen	Controls	Frozen	Controls	Frozen	Controls	Frozen	Controls	Frozen	Controls	Frozen	Controls	Frozen	Controls	Frozen
Hardy:																		
Turkestan	100	37	100	77	95	90	100	97	95	90	100	60	90	70	90	83	55	17
Grimm	100	17	100	83	100	83	100	80	100	87	95	60	100	80	90	43	70	63
Ladak	100	13	100	83	100	83	100	83	100	87	90	63	90	80	70	43	25	20
Average	100	22	100	81	98	85	100	87	98	88	95	61	93	77	83	56	50	33
Mid hardy:																		
South Dakota	100	50	95	53	95	93	100	93	100	73	75	60	90	60	75	43	40	20
Nebraska	100	23	100	47	100	90	95	80	95	80	80	57	95	73	30	33	30	33
Utah	90	7	90	27	100	87	100	77	85	83	80	63	80	53	90	63	55	40
Average	97	27	95	42	98	90	98	83	93	79	78	60	88	62	65	46	42	31
Nonhardy:																		
Italian	90	17	100	57	100	63	100	67	100	97	25	23	25	23	35	17	20	13
South African	95	23	100	47	100	90	95	87	80	70	55	3	60	43	----	----	----	----
Arizona	100	3	100	73	100	37	100	40	100	77	30	3	30	37	65	17	----	----
Average	95	14	100	59	100	63	98	65	93	81	37	10	38	34	50	17	20	13
Grand average	97	21	98	61	99	79	99	78	95	83	70	44	73	58	68	43	42	29

[a] 20 plants were used as controls and 30 plants of each alfalfa were frozen.

PERCENTAGE OF SURVIVAL OF CONTROL PLANTS

Regardless of the kind of alfalfa, the control plants dug in the fall survived the operations of digging and transplanting in the greenhouse, the percentage of survival being near 100 in all instances on

[4] Through the courtesy of the tabulating machines division of the International Machines Corporation, this method of analysis was made possible. The writers wish especially to acknowledge their indebtedness for the facilities made available by the branch offices of this company at Des Moines, Iowa, and Omaha, Nebr.

all five dates during the fall of 1928. Only occasional injuries were noted on any of these plants when they were dug up two months after they were planted in the greenhouse. On the whole, there were no marked differences in the percentage of survival among the controls of the nine alfalfas.

As the ground was frozen from December 22, 1928, until March 10, 1929, no plants were dug and artifically frozen during this period. The frost line reached a depth of almost 2 feet during the winter, and when the plants were dug in the field on March 12, there still remained considerable frost in the soil at a depth of 7 inches.

An entirely different set of results was obtained with the control plants in the spring. (Table 4 and fig. 3.) The plants of the hardy alfalfas showed a rather high percentage of survival on the first two dates in the spring, while there was a slight reduction in survival

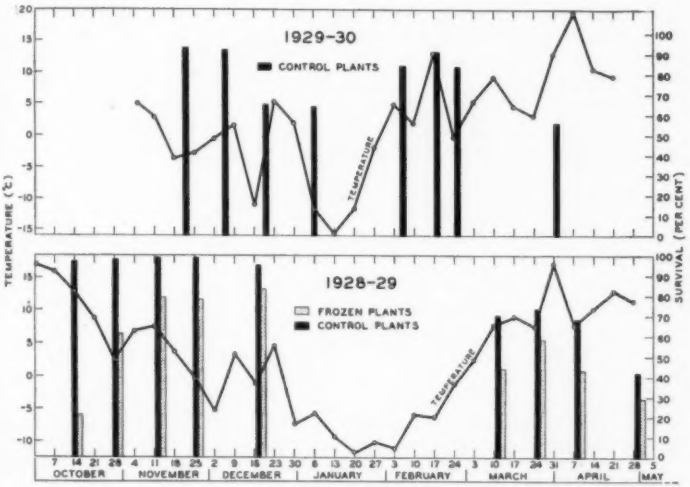

FIGURE 3.—Weekly mean temperatures for the seasons of 1929-30 and 1928-29, and the average percentage of survival of all nine alfalfas, including the control plants for the two seasons and those frozen the first season at various dates

among the plants brought in April 9 and considerable mortality on the last date. The plants of the mid hardy alfalfas behaved in a similar manner except that the percentage of survival on all corresponding dates was somewhat lower. Among the nonhardy alfalfas the mortality was high on all dates in the spring, indicating that some of the plants used in these tests had been so injured by cold in the field that they failed to recover and grow when transplanted in the greenhouse.

PERCENTAGE OF SURVIVAL OF ARTIFICIALLY FROZEN PLANTS

On October 15 a low percentage of survival was obtained with all the alfalfas in the artificial freezing test. (Table 4 and fig. 3.) The plants were still making some growth at this time and were therefore in an unhardened condition. By October 29 the hardy alfalfas were

apparently much more hardened, since a higher percentage of survival was obtained. On the succeeding dates only a slightly higher consecutive percentage of survival of the hardy alfalfas was recorded in the artificial freezing tests, indicating that while the plants were becoming more hardened, the process was more gradual.

The mid-hardy alfalfas did not harden as rapidly as the hardy alfalfas, and it was not until November 13 that they were hardened off sufficiently to increase survival appreciably in the freezing tests.

As is well known, the nonhardy alfalfas usually continue growth until killed back by low temperatures. Thus, the percentage of survival in the nonhardy alfalfas was fairly low in October and only gradually increased in November and December.

Lower percentages of survival were obtained with all alfalfas exposed to artificial freezing in the spring. It is apparent, however, that the hardy alfalfas survived better and for longer periods in the spring than the nonhardy alfalfas.

Because of the variability noted no attempt will be made to compare the behavior of the individual alfalfas within the same group in the artificial freezing tests. It is clear, however, that marked differences were obtained between alfalfas of different groups. Some of the factors responsible for this variability will be suggested under the section dealing with the relation of plant characters to survival.

Time of Killing in the Field

In an effort to determine what actually happens to alfalfa plants in the field during the fall, winter, and spring, 1-year-old plants of six alfalfas were brought into the greenhouse and planted on eight different dates during the season of 1929–30. The results, as shown in Table 5, are very similar to those for the control plants in 1928–29 (Table 4), namely, a high percentage of survival in the fall and winter and a high percentage of mortality in the April test. The rather low percentage of recovery obtained on December 20 and January 6 might be due to the fact that the ground was frozen solid and was thawed rapidly in order to release the plants from the soil. That it was not due to environmental conditions in the field is shown by the high percentage of survival in February, after most of the minimum temperatures for the winter were over. The mean weekly temperature for the seasons 1928–29 and 1929–30, together with the percentage of survival of field plants in the greenhouse, are shown in Figure 3. As in the previous experiment, all alfalfas showed a high percentage of recovery in the greenhouse during the fall, but in the spring the percentage of recovery was much lower, depending on the relative hardiness of the individual alfalfas.

TABLE 5.—*Percentage of field plants of six alfalfas that survived transplanting into the greenhouse at eight periods during the season of 1929–30*

Kind of alfalfa	Nov. 22		Dec. 6		Dec. 20		Jan. 6		Feb. 6		Feb. 18		Feb. 25		Apr. 1	
	Total plants	Survival	Total plants	Survival	Total plants	Survival	Total plants	Survival	Total plants	Survival	Total plants	Survival	Total plants	Survival	Total plants	Survival
	Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Hardy:																
Grimm	86	99	100	97	100	75	100	93	88	98	100	97	100	92	100	80
Turkestan	78	90	91	86	100	66	90	56	75	99	100	94	100	83	100	47
Average		95		92		71		75		99		96		88		64
Mid hardy:																
South Dakota	76	86	71	90	100	54	90	57	90	82	90	92	100	86	100	54
Utah	100	95	83	92	100	85	100	69	100	90	100	91	100	85	100	66
Average		91		91		70		63		86		92		86		60
Nonhardy:																
California	100	91	78	94	100	65	100	58	100	77	100	90	100	83	100	40
Arizona	96	98	104	92	100	47	100	52	100	60	100	79	100	73	100	46
Average		95		93		56		55		69		85		78		43
Grand average		93		92		65		64		85		91		84		56

TABLE 6.—*Average percentage of alfalfa plants of the nine sorts that survived three exposures to a temperature of −18 C. with the percentage injured of the survivals, at three periods in the fall of 1928 and three in the spring of 1929*

Kind of alfalfa	Percentages of survival and of injury after length of exposure indicated															
	Fall, 1928								Spring, 1929							
	None		0.5 hour		1 hour		2 hours		None		0.5 hour		1 hour		2 hours	
	Survival	Injured of survivors	Survival	Injured of survivors	Survival	Injured of survivors	Survival	Injured of survivors	Survival	Injured of survivors	Survival	Injured of survivors	Survival	Injured of survivors	Survival	Injured of survivors
Hardy:																
Turkestan	98	10	99	27	92	38	64	83	93	50	75	83	71	89	22	92
Grimm	100	0	100	18	83	41	37	92	95	41	82	60	61	72	20	88
Ladak	100	2	96	27	84	68	39	85	83	54	79	67	62	55	38	79
Average	99	4	98	24	86	49	47	87	90	48	79	70	65	72	27	86
Mid hardy:																
South Dakota	98	7	98	26	87	48	42	94	80	85	74	83	54	81	17	96
Nebraska	97	5	98	28	83	30	45	94	68	74	64	59	54	79	9	100
Utah	95	5	98	44	82	73	30	86	83	54	64	74	60	70	17	97
Average	97	6	98	33	84	50	39	91	77	71	67	72	56	77	14	98
Nonhardy:																
Italian	100	2	97	23	76	61	31	92	28	86	22	90	20	100	2	100
South African	92	2	92	50	82	78	26	97	58	54	40	65	23	62	7	100
Arizona	100	10	96	40	50	87	16	82	42	100	17	100	19	100	5	100
Average	97	5	95	38	69	75	24	90	43	80	26	85	21	87	5	100

RELATION OF PERCENTAGE OF PLANTS INJURED TO PERCENTAGE THAT SURVIVED

Table 4 shows only the percentage of survival of the plants exposed for one hour at −18° C. In Table 6 the average results obtained for each of the last three dates in the fall and the first three in the

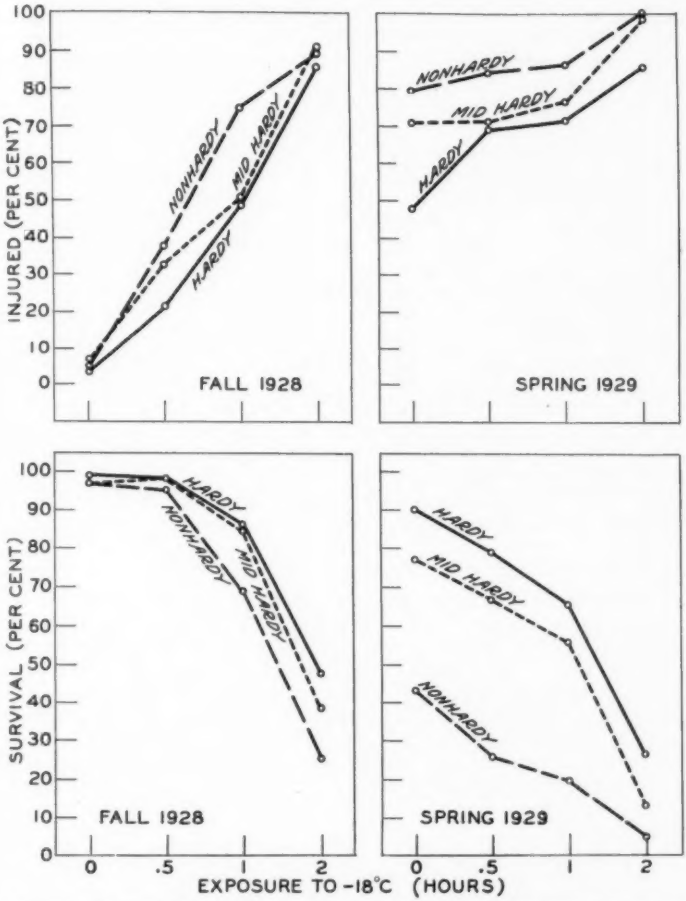

FIGURE 4.—Relation of percentage injured of the hardy, mid hardy, and nonhardy alfalfas to the percentage which survived the various exposures to a temperature of −18° C. in the fall of 1928 and spring of 1929.

spring at all exposures are given. There is also included the percentage of surviving plants that showed cold injury.

As previously mentioned, mortality among the control plants was low in the fall, and much higher in the spring. Of the plants that survived only a small percentage showed injury in the fall, whereas a large percentage showed injury in the spring. There was not only a larger number of plants surviving but there was also a smaller number

injured among the hardy than among the mid-hardy alfalfas. The lowest percentage of surviving plants and the highest percentage of injured occurred among the nonhardy alfalfas.

An exposure of one-half hour to a temperature of $-18°$ C. caused little if any mortality in the fall, although some cold injury resulted. In the spring, however, even a short exposure of plants which had survived the low temperatures of the winter in the field reduced the percentage of survival somewhat and materially increased the percentage of injury. A well-marked difference both in survival and injury was observed among the three groups of alfalfa. One hour's exposure resulted in a much higher percentage of mortality and also a higher percentage of injury both in the fall and spring.

The percentage of survival among the plants exposed for two hours was naturally low, and more of the plants that survived were injured. The percentage of surviving plants was higher and the percentage of injured plants was lower in the fall than in the spring. The differences between the groups were also marked, as can be seen from Figure 4. Combining all alfalfas in all comparisons $N=81$, the correlation coefficient between the percentage of survival and the percentage injured is -0.8458 ± 0.0220. Taking the three hardy, the three mid hardy, and the three nonhardy alfalfas separately, making $N=27$ in each instance, the correlation coefficients are -0.8090 ± 0.0470, -0.8750 ± 0.0316, and -0.8469 ± 0.0368, respectively. The decided correlation between the percentage of plants that survived and the percentage of plants injured can also be seen in Table 6 and Figure 4, from which the following rule may be deduced: As the percentage of survival decreases, irrespective of the sort of alfalfa, the percentage of injured plants increases in direct proportion.

RELATION OF PLANT CHARACTERS TO SURVIVAL

In order to obtain information on the vigor of the plants used in the tests, notes on each plant were made prior to freezing. These notes included the diameter of the roots just below the crowns, the number of buds and stems, field injuries present on the roots or crowns, and, finally, a rough characterization of the general condition of the plant. By comparing the behavior of each plant after freezing with its behavior before freezing, it was thought that some correlation between the two conditions of the plant and its ability to withstand low temperatures might be ascertained.

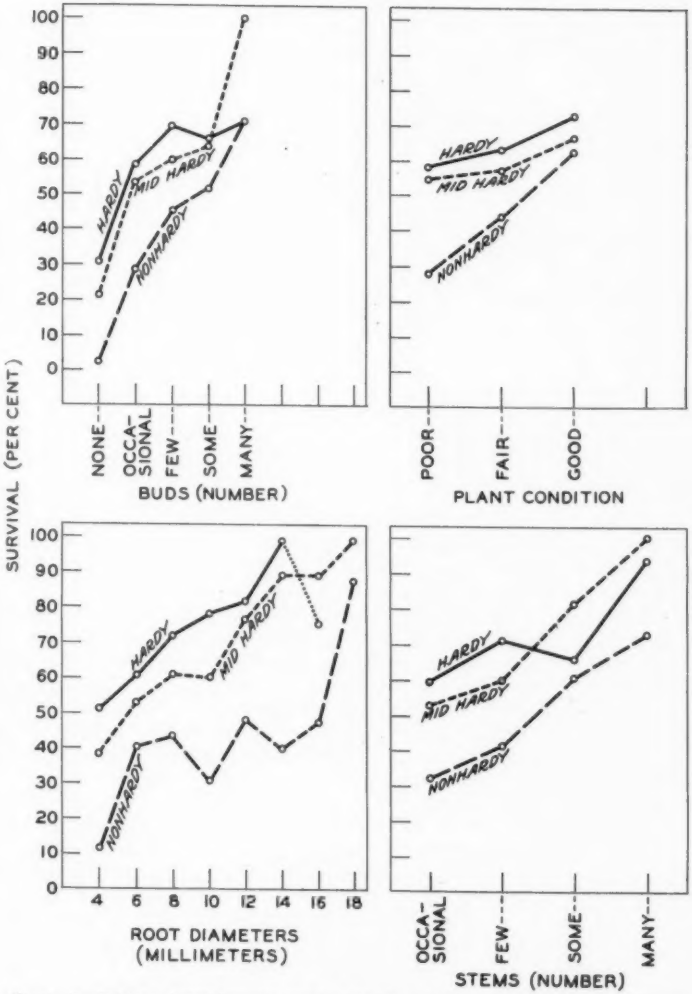

FIGURE 5.—Relation of root diameter, number of buds and stems, and general condition of the plant to the percentage of survival at all exposures and dates to a temperature of −18° C.

TABLE 7.—*Relation of root diameter, number of buds and stems, and the general condition of alfalfa plants of different kinds to survival at all exposures to a temperature of −18° C. at seven periods during the season of 1928–29*

Item	Survival of alfalfa plants					
	Hardy		Mid hardy		Nonhardy	
	Total plants	Survival	Total plants	Survival	Total plants	Survival
Root diameters:	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
4 mm	100	51	48	38	16	12
6 mm	970	61	988	54	606	40
8 mm	524	73	529	61	391	44
10 mm	159	79	191	61	217	31
12 mm	108	82	109	77	281	49
14 mm	10	100	12	89	78	40
16 mm	11	75	11	89	109	48
18 mm	0	0	1	100	42	88
Buds:						
None	42	31	38	22	82	3
Occasional	457	59	512	54	443	29
Few	1,012	70	1,132	60	881	45
Some	299	66	200	65	266	52
Many	72	71	7	100	68	72
Stems:						
Occasional	825	59	908	53	461	32
Few	916	72	917	61	1,048	42
Some	133	67	62	82	212	61
Many	10	94	2	100	16	73
Plant condition:						
Poor	457	58	537	55	562	28
Fair	1,052	63	1,054	57	859	44
Good	373	73	298	67	312	63

ROOT DIAMETERS

The diameters of the roots were taken with a wedge-shaped caliper just below the crowns prior to freezing. The data, which are presented in Table 7 and Figure 5, show that, irrespective of the sort of alfalfa, there is a direct relation between the diameter of the root and the survival of the plant. With roots of the same diameter, the chances for survival are greatest in the hardy alfalfas. Within the same sort of alfalfa a much higher percentage of survival was obtained with roots of large diameter. No doubt some of the variability in the artificial freezing tests may be accounted for in part by the size of the root, especially among those alfalfas in which there was greater variability in the size of the roots.

NUMBER OF BUDS

It is apparent from Table 7 and Figure 5 that there is a distinct relation between the number of buds present on plants prior to freezing and the percentage of plants that survived after freezing. The more buds there are on plants, the greater are their chances for survival. Especially is this true in the mid-hardy and nonhardy groups. Of a total of 82 plants without buds in the nonhardy group only 3 per cent survived, whereas of the 68 plants with many buds 72 per cent survived.

NUMBER OF STEMS

In general, there is a distinct relation between the number of stems per plant and the ability of the plants to withstand low temperatures. (Table 7 and fig. 5.) This relation is most marked and consistent in the mid-hardy and nonhardy groups.

Plant Condition

For recording the general condition of the plants, they were separated into three lots on the basis of their appearance, including root, crown, buds, and stems. The results, presented in Table 7 and Figure 5, show that the better the condition of the plant the greater are its chances of surviving artificial freezing. The low percentage of survival of the plants in poor condition and the high percentage of survival of plants in good condition in the nonhardy group is quite marked.

The data presented in Table 7 allow statistical analysis by the χ^2 test of independence (*1*). This method does not measure the degree of association between one class and another, but it does test whether the observed departures from random distribution are or are not of a magnitude ascribable to chance. The values of P are not tabulated, it being sufficient to state that in all sets the value of P was less than 0.01, which indicates probabilities of 100 to 1 or more that the deviation from independent grouping is not due to chance. From the data summarized in Table 7, an example is presented in Table 8 combining the three exposures for all dates of Ladak alfalfa, and separating the plants into healthy, injured, and dead.

Table 8.—*Example showing manner is which statistical analysis of data by the χ^2 test of independence was made*

Plant condition	Number of healthy plants		Number of injured plants		Number of dead plants		Total plants
	Observed	Calculated	Observed	Calculated	Observed	Calculated	
Poor	40	52.3	47	47.7	64	51.1	151
Fair	119	124.6	125	113.7	116	121.7	360
Good	59	41.8	27	37.6	33	40.2	119
Total	218		199		213		630

χ^2=19.154. P=less than 0.01.

The departure from independence is significant and is due to the relatively large number of dead plants and the small number of healthy plants in the poor class as compared to the number of each in the good class. The same calculation made on the control plants of the same alfalfa gives a value for χ^2 of 6.1814, which with N=4 and P= between 0.10 and 0.20, indicates that the fit to independent grouping is quite good. In other words, the survival in the controls is about the same whether the plants are poor, fair, or good, but when the plants are frozen the distribution is distinctly altered and no longer follows a random distribution within the groups.

Types of Winter Injury

For convenience, the type of cold injuries observed in the freezing tests may be roughly classified into two groups, namely, crown and root. Crown injuries include any damage to the buds, to young shoots, or to the crown. Root injuries involve the phloem, cambium, or xylem. The term "internal root injuries" includes discoloration, splitting, and any other disorganization of the xylem.

A third type of injury, the cracking or splitting of the phloem and xylem, will be discussed later as it generally follows phloem injury and is not directly due to freezing.

Crown, phloem, and internal root injuries have recently been described by Jones (*2*) and Weimer (*8*). The types of injury observed by the present writers were, for the most part, of the same nature. In general, it may be said that the crown and phloem injuries or a combination of the two were most evident. Possibly the fact that all plants were examined eight weeks after freezing may account for the absence of what Weimer refers to as heart rot, although when examined some plants showed various degrees of discoloration in the inner root xylem. Very little evidence of internal root splitting was observed when the plants were examined eight weeks after the freezing tests. In preliminary tests made to determine the amount of splitting immediately after freezing, it was found that splitting could be induced by a slight pressure exerted with a razor blade in cutting the roots. The point of weakest stress in the frozen roots was along the medullary rays, as reported by Jones (*2*) and Weimer (*8*). However, the large amount of internal splitting reported by them was not observed by the writers.

An effort was made to correlate the types of injury with the time of the year in which freezing tests were made. Thus, in Table 9 is listed the percentage of plants that showed crown or phloem injury or both in the fall and spring tests. It is evident that the plants that survived the fall tests had a greater amount of crown than of phloem injury. The percentage of crown and phloem injury naturally increased with the length of exposure. In the spring, while there appears to be a greater amount of phloem injury, there is also considerable crown injury, with a large percentage of plants showing both. There does not appear to be any great varietal difference in response to cold injury, the same types being found on all alfalfas. However, as in previous experiments, the percentage of injured plants was highest among the nonhardy alfalfas. The percentage of injured plants was much lower in the fall than in the spring.

In the fall alfalfa plants appear to be more subject to crown injury, although phloem injury alone or in combination with crown injury occurs. A much higher percentage of both kinds of injury prevailed in the spring, and the extent and severity of the injuries were much more pronounced. Even the control plants not subjected to freezing showed fairly high percentages of cold injury in the spring. The large percentage of plants exposed to freezing in the spring that showed both crown and phloem injury accounts for the relatively small percentage that showed only crown or only phloem injury. There do not appear to be any differences in the types of cold injury in the various alfalfas, although the extent and severity of the injuries are directly related to the relative hardiness of the alfalfas.

As mentioned previously, another type of injury, especially prevalent on the plants in the spring, was found, apparently as the indirect result of artificial freezing. This injury consisted of cracks of different depths on the roots of the plants, ranging in length from a few millimeters to several centimeters. For the most part, these cracks appeared to have their inception in injured phloem tissue. Occasionally however, a plant was found with root cracking in the absence of phloem injury.

TABLE 9.—*Percentage of the various types of injuries on alfalfa plants of nine sorts that survived 3 exposures to a temperature of −18° C. at three periods in the fall of 1928 and three in the spring of 1929*

Kind of alfalfa	Percentage of survivors having type of injuries and exposed to freezing for periods indicated											
	Fall, 1928											
	Crown injuries				Phloem injuries				Crown and phloem injuries			
	None	0.5 hour	1 hour	2 hours	None	0.5 hour	1 hour	2 hours	None	0.5 hour	1 hour	2 hours
Hardy:												
Turkestan	3	11	9	33	5	6	8	13	2	10	19	30
Grimm	0	12	27	39	0	2	10	7	0	0	3	32
Ladak	0	12	21	44	2	8	19	9	0	7	23	29
Average	1	12	19	39	2	5	12	10	1	6	15	30
Mid hardy:												
South Dakota	0	15	28	5	5	5	6	31	2	9	20	51
Nebraska	5	21	26	28	0	5	9	5	0	2	13	56
Utah	5	19	39	37	0	12	14	0	0	7	18	33
Average	3	18	31	23	2	7	10	12	1	6	17	47
Nonhardy:												
Italian	0	14	27	32	0	1	12	0	0	8	20	43
South African	5	31	40	24	0	3	10	12	0	10	21	52
Arizona	3	24	39	30	7	11	18	20	0	6	25	20
Average	3	23	35	29	2	5	13	11	0	8	22	38
Grand average	2	18	28	30	2	6	12	11	1	7	18	38

Kind of alfalfa	Percentage of survivors having type of injuries and exposed to freezing for periods indicated											
	Spring, 1929											
	Crown injuries				Phloem injuries				Crown and phloem injuries			
	None	0.5 hour	1 hour	2 hours	None	0.5 hour	1 hour	2 hours	None	0.5 hour	1 hour	2 hours
Hardy:												
Turkestan	14	16	19	8	16	4	6	0	37	51	61	64
Grimm	5	1	10	11	36	18	17	0	5	21	24	44
Ladak	4	20	12	10	24	11	8	14	22	25	26	41
Average	8	12	14	10	25	11	10	5	21	32	37	50
Mid hardy:												
South Dakota	13	12	12	14	27	15	0	0	34	35	45	48
Nebraska	4	12	12	18	26	22	27	9	28	29	29	18
Utah	11	16	14	14	21	12	2	9	23	35	43	43
Average	9	13	13	15	25	16	10	6	28	33	39	36
Nonhardy:												
Italian	0	3	0	0	3	8	8	0	38	38	65	25
South African	0	0	12	0	3	14	6	0	35	14	53	100
Arizona	0	0	4	0	18	4	19	0	58	37	46	100
Average	0	1	5	0	8	9	11	0	44	30	55	75
Grand average	6	9	11	8	19	12	10	4	31	32	44	54

95073—32——2

This external cracking of the root is not to be confused with the internal splitting of the root tissues discussed previously and fully described by Jones (*2*) and Weimer (*8*). So far as can be determined, no mention of this type of injury has been made by previous investigators, although it is not entirely clear from Jones's (*2*) histological study whether or not he had this type under observation.

TABLE 10.—*Percentage of alfalfa plants of nine sorts surviving a temperature of −18° C. at eight periods during the dormant season of 1928–29 that showed definite cracking of the roots*

Kind of alfalfa	Percentage of survivors with cracking							
	Oct. 29	Nov. 13	Nov. 26	Dec. 18	Mar. 12	Mar. 26	Apr. 9	Apr. 30
Hardy:								
Turkestan	0	1	0	4	39	38	35	35
Grimm	0	5	1	2	45	15	29	33
Ladak	3	1	0	1	33	24	53	52
Average	1.0	2.3	.3	2.3	39	26	39	40
Mid hardy:								
South Dakota	0	0	0	0	41	42	65	61
Nebraska	0	0	0	2	48	38	48	51
Utah	0	0	0	2	24	20	38	69
Average	0	0	0	1.3	38	33	50	60
Nonhardy:								
Italian	0	0	1	0	58	55	63	85
South African	0	0	0	8	62	37	.....	.....
Arizona	0	0	0	4	92	91	100	.....
Average	0	0	.3	4.0	71	61	82	85
Grand average	.3	.8	.2	2.5	49	40	54	55

In Table 10 is listed the percentage of alfalfa plants surviving a temperature of −18° C. that showed definite cracking of the roots. It will be noted that little or no cracking occurred in the fall. What little cracking was present is apparently not related either to the kind of alfalfa, the length of exposure to low temperatures, or the date of the tests. In other words, cracking does not occur consistently in the fall. It can readily be seen from the large percentage of plants with cracked roots that this type of injury is primarily associated with early spring conditions, when the plants lose their hardiness and rapid growth processes are being initiated. There does not seem to be any varietal response to cracking, but it appears to be related directly to the extent and severity of phloem injury present.

In order to find how long after the plants were frozen cracking would become evident on the roots, eight hundred 3-year-old plants of each of the two alfalfas Grimm and Ladak were brought in from the field in November, 1929, and again in February and late March, 1930. Four hundred plants served as controls, and a like number were exposed for one hour to a temperature of −18° C. After freezing they were planted in the greenhouse, and 100 of the controls and frozen plants, respectively, were lifted and examined every two weeks for a period of two months.

No cracking was evident on the plants frozen during November, cold injury being limited to the crown and root. Typical cracking was found on the plants frozen in February after they had been

growing in the greenhouse for six weeks. In all instances cracking was connected with either field or artificial cold injury on the roots. The roots of badly cold-injured plants did not show any cracks. A fairly high percentage of the plants which survived and were injured had definite cracks: Grimm, controls 34.7 per cent, frozen 66.7 per cent; Ladak, controls 31.1 per cent, frozen 31 per cent. It was apparent from an examination of the cracked roots that the cracks were due primarily to a growth phenomenon. As new root growth was resumed the dead areas of phloem did not respond, so that an unequal stress occurred, resulting in the cracking of the tissues. The third set of plants, frozen in late March, showed no evidence of cracking, although cold injuries were more numerous and severe.

In the spring of 1928 the senior writer observed cracking on roots in the field. In connection with wilt studies in a number of alfalfa fields in the Platte Valley, Nebr., plants were dug up every month from an area of 4 square feet. Of the plants dug in early April, a number showed typical winter injury but were otherwise normal. However, early the following month many injured plants had large cracks on the roots. In June plants from these fields showed no definite cracking, but there was evidence that the cracks had grown together.

Judging from the experimental tests and field observations, the phenomenon of external root cracking is first of all related to phloem injury, and probably occurs from four to six weeks after the rapid growth processes of the plants are initiated in the early spring. These wounds finally close up and heal as growth continues.

Notes taken on individual plants before they were frozen included rots of various kinds. These were classified as follows: Crown, collar, heart and surface. Several Fusaria have been isolated from these various types of rots. Surface rot of the roots has been found to be caused by Rhizoctonia. From 30 to 38 per cent of plants of South Dakota, Turkestan, Ladak, and Grimm had a slight to moderate amount of surface rot. While there was a slightly greater amount of the crown, collar, and heart rots on the nonhardy plants, the percentage of plants with one or more rots was small. Likewise, the number of plants with winter-injury wounds was small but was greatest in the nonhardy alfalfas. The total number of plants of each alfalfa with Fusarium rots and winter injury was so small that it has been impossible to determine the influence of these rots on the subsequent behavior of the plants in the artificial freezing tests. In the case of surface rot where rather high percentages of plants of certain alfalfas were infected, surface rot, as such, did not make the plants more susceptible to cold injury. Probably the Fusaria, which are saprophytes, or, at best, weak parasites, follow injuries in the field.

RATE OF RECOVERY OF SURVIVING PLANTS

In order to determine whether there was any relation between cold injury and the subsequent recovery of plants, a record of the plants that recovered was made 2, 4, and 8 weeks after freezing. The data, including the results obtained at eight periods during the season, are presented in Table 11. The results show that, irrespective of the kind of alfalfa, there is a direct relation between the extent and severity of cold injury and the ability of the plants to recover. In other words, the greater the cold injury, the slower are the plants to recover and resume growth, as shown by the decreasing percentages

of recovery at the 2-week period with increased freezing exposure. There is no consistent difference in the rate of recovery between the hardy, mid hardy, and nonhardy alfalfas. A small percentage of the plants died after initial recovery, and here again it was evident that they died because of the extent of the cold injury sustained.

TABLE 11.—*Average rate of recovery of alfalfa plants that survived three exposures to a temperature of −18° C. at eight periods during the season of 1928–29*

Kind of alfalfa	Percentage of survivors recovering after indicated length of exposure											
	None			0.5 hour			1 hour			2 hours		
	After 2 weeks	After 4 weeks	After 8 weeks	After 2 weeks	After 4 weeks	After 8 weeks	After 2 weeks	After 4 weeks	After 8 weeks	After 2 weeks	After 4 weeks	After 8 weeks
Hardy:												
Turkestan	86	10	4	75	20	5	64	24	12	34	38	28
Grimm	77	20	3	72	23	5	68	23	9	45	31	24
Ladak	85	12	3	71	21	8	58	30	12	32	40	28
Average	83	14	3	73	21	6	63	26	11	37	36	27
Mid hardy:												
South Dakota	86	11	3	68	24	8	46	35	19	23	25	52
Nebraska	81	11	8	65	20	15	58	23	19	30	25	45
Utah	81	11	8	67	23	10	54	31	15	62	25	13
Average	83	11	6	67	22	11	53	30	18	38	25	37
Nonhardy:												
Italian	82	13	5	66	23	11	61	20	19	69	23	8
South African	77	16	7	67	17	16	47	31	22	47	31	22
Arizona	84	11	5	69	21	10	48	33	19	29	24	47
Average	81	13	6	67	20	12	52	28	20	48	26	26
Grand average	82	13	5	69	21	10	56	28	16	41	29	30

The data in Table 11 were rearranged in such a way as to show whether there was any outstanding difference between the recovery of the plants frozen in the fall and those frozen in the spring, but since no decided difference was found the data are not presented. It should be noted, however, that there appeared to be a slower recovery of the plants injured by cold in the late fall, which fact may be related to the depressing effect of the length of day under which the plants were grown in the greenhouse rather than to the effect of freezing.

RELATION OF SUBSEQUENT NEW GROWTH TO COLD INJURY

In order to determine what effect cold injury has on the subsequent recovery of the plants and on the amount of new growth produced, records were taken of the number of stems and the height of the new growth of all the plants that survived at the end of eight weeks after freezing. The number of stems per plant was found to be a good index of cold injury, but the height of the new growth was not, since when only a few new shoots were produced, the height was greater than when many new shoots were present. In the latter case the total amount of new growth was greater. In all plants in which cold injury was observed, the average number of stems was less than in the uninjured plants.

To ascertain the total amount of growth after freezing, four alfalfas were exposed to a temperature of −18° C. for one hour in November,

1929, and again in March, 1930. The plants were of the same age and were prepared similarly to those in the other freezing experiments. After growing in the greenhouse for eight weeks, the plants were divided into three lots. Healthy plants with no injuries whatever constituted the first lot; plants which had old field injuries but no cold injuries from artificial freezing the second; and plants with evident injuries produced by artificial freezing the third lot. The control plants were also divided into the first two classifications. Finally, the new growth was weighed three hours after cutting and the average green weight of the tops per plant was determined. In the spring test the roots also were weighed. The results are recorded in Table 12.

TABLE 12.—*Average weight in grams per plant, tops and roots, of four kinds of alfalfa which survived an exposure of one hour to a temperature of −18° C.*

Kind of alfalfa	November 6, 1929					March 28, 1930									
	Average weight of tops					Average weight of tops					Average weight of roots				
	Controls		Frozen			Controls		Frozen			Controls		Frozen		
	Healthy	Field injuries	Healthy	Field injuries	Cold injuries	Healthy	Field injuries	Healthy	Field injuries	Cold injuries	Healthy	Field injuries	Healthy	Field injuries	Cold injuries
Grimm	4.1	1.7	3.7	1.8	0.9	4.8	1.9	5.3	1.8	1.7	6.3	5.5	5.2	5.0	4.4
Ladak	4.5	2.2	4.2	2.0	1.0	5.9	1.8	5.9	2.2	.8	7.2	4.5	7.9	5.0	4.6
Turkestan	4.9	2.5	4.7	2.8	1.1	6.6	2.9	5.9	1.8	.9	10.5	8.0	11.1	7.8	8.4
Nebraska	6.5	4.5	4.9	3.7	.9	6.1	1.4	7.1	1.8	.8	11.2	7.2	11.3	6.6	7.6
Average	5.0	2.7	4.4	2.6	1.0	5.9	2.0	6.1	1.9	1.1	8.8	6.3	8.9	6.1	6.3

There is very little difference between the weight of the tops of the healthy control plants and of the healthy frozen plants. There is likewise little difference between the weight of the tops of the field-injured controls and of the field-injured frozen plants, although the weight of the tops of the field-injured plants is approximately only half as great as that of the healthy plants. The weight of the tops of plants that were injured by artificial freezing was very small both in the fall and spring, being less than that of any of the other classes.

Little or no difference was found between the weight of the roots of the healthy controls and of the healthy frozen plants. Similarly, no difference was found between the weight of the roots of plants injured in the field and of plants injured by artificial freezing, although the weight of the roots of these plants was somewhat smaller than that of healthy plants. Weight of roots is not an index to the extent and severity of cold injury. Cold injury, however, is reflected directly in new growth, the amount depending primarily on the extent and severity of the injury to the crown and root tissues. No doubt the yield from injured plants in the field is reduced in proportion to the amount of injury present in the crown and roots.

DISCUSSION

Investigators are generally agreed that the moisture content of plants is highest in the summer, decreases to a minimum in winter, and rises rapidly in the spring after the rest period is broken and growth is resumed. The moisture content of the alfalfa plants (roots and crowns) used in these tests showed generally the same trend. The only explanation that can be offered for the decreasing moisture content of the nonhardy alfalfas in the spring is that some of the plants used for determining the moisture content had been injured by the low temperatures of the winter, but when taken for the sample had not yet shown the effects of this condition. The low percentage of survival among the control plants of the nonhardy alfalfas in the spring tends to substantiate this conclusion. There appeared to be no consistent differences in the moisture content of hardy and nonhardy alfalfas in the fall, although Newton (*4*, *5*) found that the hardier varieties of wheat in general contain less moisture in the leaves during the dormant season.

The use of plants brought in from the field and planted directly in the greenhouse with others exposed to artificial freezing serves to differentiate the influence of field environmental factors on the plant from those produced by artificial freezing, and brings out a number of interesting points. A large percentage of all the control plants tested on the five dates in the fall of 1928 survived, including plants in an unhardened state, up to the time that the ground was frozen. However, in the spring there was a progressive decrease in the number of surviving plants as the season advanced.

Similar results were obtained in 1929–30 by simply bringing in plants from the field at different dates during the season and planting them in the greenhouse. Again a high percentage of the plants survived even when transplanted from the field to the greenhouse as late as February, but in April the mortality was much greater.

The question naturally arises, why is it that plants brought in from the field in late February and early March and planted in the greenhouse give a high percentage of survival, even the nonhardy alfalfas, whereas plants brought in after the rest period is broken show an increasingly greater mortality in April, depending on the hardiness of the alfalfa. The answer to this question may be found in the relation of cold-injured plants to their environment. Under the adverse environmental field conditions prevailing at the time the rest period is broken and growth is resumed in the spring, the plants weakened or injured by the low temperature of the winter are unable to continue normal growth and finally succumb. Similar field plants brought in and planted in the greenhouse before the rest period is broken are able to survive under optimum growing conditions, but plants taken in after growth is resumed in the field are unable to survive even under optimum growing conditions.

While field observations indicated that some plants did not recover in the field, the majority of the injured plants died after growth was resumed. Depending on the severity of the winter, the proportion of the plants killed outright may be larger than was found under the above conditions. In neither 1928–29 nor 1929–30, did temperatures low enough to cause killing occur after early March. (Fig. 3.)

Steinmetz's (*6*) observations in Minnesota during two years are in accord with those of the writers in Nebraska in showing that very little killing occurs during the winter and dead plants are not observed until periods of thaws in the early spring. Weimer (*7*) found dead plants during December, January, and February in Kansas. There is little doubt that temperatures high enough to initiate growth processes in alfalfa prevail during the ordinary Kansas winter for periods long enough to produce the same effect on plants as that observed later in the season in Nebraska and Minnesota. The time required for breaking the rest period of alfalfa plants and initiating growth activities is extremely short, a matter of a few days, whereas the hardening process in the fall is a matter of weeks, hardening under Nebraska conditions continuing, in 1928, into late December.

The artificial freezing tests in the fall bring out the fact that the percentage of survival is low with all alfalfas when frozen in an unhardened condition. The hardy alfalfas, however, not only become dormant earlier in the fall, but they harden off more rapidly, whereas the nonhardy alfalfas grow on into the fall until the tops are killed back by low temperatures, and the hardening process is much slower. The mid-hardy alfalfas go into dormancy somewhat later than the hardy alfalfas, but become hardened more rapidly than the nonhardy alfalfas. The hardening process in the fall appears to be cumulative over a rather long period.

The percentage of survival in the spring freezing tests was much lower for all the alfalfas. However, the hardy alfalfas survived better and over a longer period in the spring than the nonhardy alfalfas. By the last of April the plants were again in an unhardened condition. It is apparent that the hardy alfalfas retain their hardiness longer in the spring than the nonhardy.

Some of the obvious variable plant characters, such as size of roots, number of buds and stems, and the general condition of the plants, have a distinct bearing on survival of field plants to artificial freezing. Root diameters within any one sort of alfalfa varied from 4 to 18 mm., the number of buds and stems from none to many, and the condition of the plant from poor to good. Yet these plants were all growing under comparable field conditions.

Within the same sort of alfalfa the increased diameter of the root enhances the percentage of survival. A greater number of plants with roots of the same size survived in the hardy than in the nonhardy alfalfas. While large roots are less subject to killing than small roots within the same sort of alfalfa, usually small roots of hardy alfalfas are able to withstand freezing better than larger roots of nonhardy alfalfas. In a few instances the extremely large roots of nonhardy alfalfas were able to survive, whereas the smallest roots of hardy alfalfas were killed. Similarly, within any one sort of alfalfa the more buds and stems present and the better the condition of the entire plant, the greater is its chance of survival. All of these obvious differences in plant characters play a part in increasing the variability in cold resistance tests with field-grown plants.

Of the plants that survive in the freezing tests and in the field, a large number are injured in different degrees, depending on conditions. Winter-injured plants can be found in all alfalfa fields of any age in Nebraska, and the extent of the reduction in stand and yields resulting from these injured plants is not generally realized. In

fact the problem of winter injury in alfalfa is as important as winter killing, since even the hardier sorts are subject to winter injury.

The percentage of injured plants in the freezing tests is indirectly proportional to the percentage of surviving plants. In other words, the greater the severity of the freezing tests, the lower the percentage of surviving plants and the higher the percentage of injured plants. Although the nonhardy alfalfas have a lower percentage of survival and consequently a higher percentage of injured individuals at any definite exposure than the hardy alfalfas, all alfalfas tested are susceptible to injury.

The large variability in the percentage survival in these tests is due in a large measure to variable plant characters within any one sort of alfalfa. The prevailing environment in the field over a 2-year period serves to accentuate plant variability and at the same time to eliminate the less hardy individuals, particularly in the nonhardy sorts. The variability due to these factors must be reduced to a minimum in the development of an accurate method for determining the comparative cold resistance of alfalfas.

SUMMARY

This paper presents the results of hardiness tests obtained with 2-year-old field-grown plants of nine kinds of alfalfa, exposed to a temperature of $-18°$ C. for various periods of time on nine different dates during the season of 1928–29.

The moisture content of all alfalfas (roots and crown) decreased during the fall. The moisture content of the hardy and mid-hardy alfalfas again increased in the spring, but the moisture content of the nonhardy alfalfas remained low in March and April.

Control plants of all alfalfas brought in from the field and planted in the greenhouse showed a high percentage of survival from early fall until late winter. In April the mortality of the plants increased, depending on the hardiness of the alfalfa. It is suggested that in such years as 1929–30 low winter temperatures may be responsible for injuring plants to such an extent that after initial growth is resumed in the spring they are unable to withstand the adverse environmental field conditions prevailing at that time. The plants died in spite of the fact that freezing temperatures did not occur in the spring.

In the artificial freezing tests the percentage of survival of all alfalfas was low in the early fall. The hardy alfalfas become dormant earlier and harden more rapidly; the nonhardy alfalfas become dormant later and harden more slowly. The mid-hardy alfalfas are intermediate. The hardening process in alfalfas appears to be cumulative over a rather long period in the fall and early winter. In general, progressively higher percentages of survival of all alfalfas were obtained in late fall and early winter.

The percentage of survival in the freezing tests in the spring was much lower for all alfalfas, although it is apparent that the hardy alfalfas retained their hardiness longer in the spring than the nonhardy.

In general, within the same sort of alfalfa, the plant that has the largest root diameter, the greatest number of buds or stems, and is in the best general condition has the best chance of surviving the artificial freezing.

All alfalfas tested are susceptible to cold injury by artificial freezing. The percentage of plants showing cold injury in any one sort of alfalfa is inversely proportional to the percentage of surviving plants. The types of injury produced by artificial freezing are similar to those found on plants injured by cold in the field.

The rate of recovery of all alfalfas from artificial freezing is directly related to the extent of injuries present on the plants, as is the total amount of growth subsequently produced.

LITERATURE CITED

(1) FISHER, R. A.
1928. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 2, rev. and enl., 269 p., illus. Edinburgh and London.

(2) JONES, F. R.
1928. WINTER INJURY OF ALFALFA. Jour. Agr. Research 37:189–211, illus.

(3) PELTIER, G. L.
1931. CONTROL EQUIPMENT FOR THE STUDY OF HARDINESS IN CROP PLANTS. Jour. Agr. Research 43:177–182, illus.

(4) NEWTON, R.
1924. THE NATURE AND PRACTICAL MEASUREMENT OF FROST RESISTANCE IN WINTER WHEAT. Alberta Univ., Col. Agr. Research Bul. 1, 53 p., illus.

(5) ——— and BROWN, W. R.
1926. SEASONAL CHANGES IN THE COMPOSITION OF WINTER WHEAT PLANTS, IN RELATION TO FROST RESISTANCE. Jour. Agr. Sci. [England] 16: 522–538, illus.

(6) STEINMETZ, F. H.
1926. WINTER HARDINESS IN ALFALFA VARIETIES. Minn. Agr. Expt. Sta. Tech. Bul. 38, 33 p., illus.

(7) WEIMER, J. L.
1929. SOME FACTORS INVOLVED IN THE WINTERKILLING OF ALFALFA. Jour. Agr. Research 39:263–283, illus.

(8) ———
1930. ALFALFA ROOT INJURIES RESULTING FROM FREEZING. Jour. Agr. Research 40:121–143, illus.

THE DETERMINATION OF SAMPLE SIZE FOR DIAMETER MEASUREMENTS IN COTTON FIBER STUDIES [1]

By O. A. POPE [2]

Assistant Agronomist, Arkansas Agricultural Experiment Station

INTRODUCTION

A study of the relationships existing between the physical characteristics of cotton fiber and the quality of various yarns and fabrics manufactured from it, constitutes one of the fundamental problems in a research program concerned with the improvement of cotton varieties. Since cotton is produced almost entirely for manufacturing purposes, it is important (1) that the breeder have means of interpreting the needs of the spinner in order that he may develop new varieties which will be better adapted for specific purposes, and (2) that he have practical and dependable methods of measuring the various important fiber characteristics.

Of the properties that contribute to quality in cotton, the most important are length and fineness. In recent years much attention has been directed to other characters such as strength, convolutions, and rigidity, but length and fineness remain the dominant factors in cotton quality.

Morton,[3] in summarizing the results of spinning tests, concludes that:

> Yarn regularity depends on hair fineness to a greater extent than on any other easily measured hair property. * * * Where it is necessary or desirable to use low spinning constants, fine cottons give strengths more nearly approaching their maximum than do coarse cottons. * * * Yarn strength is apparently not at all affected by hair strength because of the masking effect of irregularity, and thus the strongest yarn will be produced not from the *strongest* cotton, but from the *finest*.

According to Barritt,[4] either fineness or length may be used as a basis for classifying the world's cottons and the same result will be obtained in each case because in a general way length and fineness are correlated. However, this correlation which appears to exist in a general way for the world's cotton, varies within relatively wide limits for individual strains and varieties. This fact makes it possible for the breeder to develop the particular combination of length and fineness that is desirable for any particular mill requirement, within, of course, reasonable limits, but at the same time it necessitates the independent evaluation of each of the properties concerned.

In the past length has been the character most extensively used in selection work. This selection for length of staple has undoubtedly resulted in considerable improvement in the quality of cotton, despite

[1] Received for publication May 11, 1931; issued January, 1932. Contribution from the department of agronomy, Arkansas Agricultural Experiment Station. Research Paper No. 258, Journal Series, University of Arkansas.

[2] The writer wishes to express his appreciation to Prof. G. W. Snedecor, of Iowa State College, for criticism of the methods of mathematical treatment used, and to his coworkers at the Arkansas experiment station for helpful suggestions and criticisms.

[3] MORTON, W. E. THE SPINNING VALUE OF RAW COTTON. Jour. Textile Inst. 21: T205-224, illus. 1930.

[4] BARRITT, N. W. THE DETERMINATION OF FINENESS OF EGYPTIAN COTTON AND ITS RELATION TO QUALITY. Empire Cotton Growing Rev. 7: 19-29, illus. 1930.

the fact that fineness has been allowed to take care of itself. Within recent years it has been shown that an increase in staple length does not necessarily effect an improvement in quality, and that within a given staple length there exists a considerable range of spinning values. The question therefore of estimating fineness independently of length is receiving increasing attention from cotton breeders.

Several measurements and methods of measuring have been suggested by different workers for determining the diameter of cotton fibers. The measurement of the diameter of the uncollapsed fiber is relatively simple [5] and offers what is probably the best genetic measure of fineness, since the diameter of the turgid fiber depends upon the size of the primary fiber envelope, and consequently is not modified by fiber-wall thickness, which depends to a large extent on environmental and nutritional factors. One practical difficulty in employing this measurement in breeding operations is the necessity of preserving the green samples in a killing and fixing reagent until it is convenient to make the microscopic examination. Another serious objection to this method in connection with spinning studies is the fact that it gives no measure of the fiber-wall thickness. The thickness of the fiber wall when considered in connection with the diameter of the uncollapsed fiber is closely related to the frequency of convolutions along the linear length of the fiber, and the regularity and frequency of the convolutions are intimately associated with drag and yarn strength. For this reason it is desirable to have a method of measuring that will evaluate the physical properties of the fiber as it is utilized by the manufacturer.

Turner,[6] in his work with Indian cottons, made comparisons between several fiber properties and the highest warp counts for which each cotton is suitable. He found that when the different cottons were arranged in order of increasing coarseness of highest suitable counts, there was a general trend in the values of any given fiber property similar to that of the counts, but in all cases there were outstanding exceptions. The parallelism was more marked in ribbon width than in fiber strength, fiber weight, number of convolutions, and fiber rigidity. However, there were so many individual exceptions to each general trend that it was concluded that no single fiber property can serve as a universal criterion to indicate the highest suitable counts into which a cotton can be spun.

The measurement of the diameter of mercerized cotton was suggested by Calvert and Harland [7] as a more reliable measure of fiber diameter than ribbon width alone. The mercerization process consists in swelling the fibers in caustic soda solution, washing in water, and drying. Since this treatment imparts a more even cross section to the fibers, diameter measurements obtained in this way should be more significant than ribbon-width determinations. The mercerization process, however, alters considerably the normal structure of the fiber in regard to convolutions, ratio of width to thickness, and drag. In view of the fact that most cotton is spun without being mercerized, it is undoubtedly more important to have a reliable measure of the

[5] BARRITT, N. W. SOME PROPERTIES OF THE CELL-WALL OF COTTON-HAIRS. Ann. Appl. Biol. 16: 438–443. illus. 1929.

[6] TURNER, A. J. THE INDIAN CENTRAL COTTON COMMITTEE TECHNOLOGICAL LABORATORY. Empire Cotton Growing Rev. 6: 215–230. 1929.

[7] CALVERT, M., and HARLAND, S. C. THE MEASURABLE CHARACTERS OF RAW COTTONS. III.—AN APPROXIMATION OF THE ORIGINAL CELL DIAMETER. Jour. Textile Inst. 15: T8–T9. 1924.

diameter characteristics of the fiber in its normal state than to depend on a constant derived from an alteration of the normal fiber. Moreover, correlations of fiber properties with spinning behavior will have a greater probable value as a basis for predicting the spinning value of a new variety than correlations derived from an altered condition which in itself is somewhat empirical and subject to variations.

The measurement of mean ribbon width, or the measurement of each fiber in two separate places, one at the flat portion of the convolution and the other at the region of twist or edge-on position, has been suggested by Barritt,[8] who, in commenting on the advantages and reliability of this method, says:

> It can be shown that the mean of these two measurements approximates more or less closely to that of the mercerized diameter, according to the amount of secondary thickening of the hair. Suppose a normally thickened hair to have a mercerized diameter of 14μ. Such a hair before wall thickening had begun would collapse to a ribbon whose widest diameter would be half the perimeter—viz., $\frac{\pi \times 14}{2}$ or 22μ. The width of the edge-on position would be, say, 2μ. The mean of these two measurements gives 12μ as the "mean" ribbon width, and this figure represents the maximum deviation from the true diameter shown by immature hairs. Thus, if all the hairs of a sample were unthickened, the reduction in the measurement of mean ribbon width could not exceed 14 per cent., whereas if, say, only one-tenth of the hairs were immature, the deviation of the mean ribbon width would be less than 2 per cent. By this system of double measurement of each hair we reduce the disturbing effect of immature and abnormal hairs to a minimum. By any other system of measurement the effect of immature hairs is to increase the mean diameter, and to a much greater extent. This is a much more serious error, since immature hairs do not behave as coarse hairs during spinning.

The data presented in the present paper were obtained from a study which had for its object the determination of the smallest number of individual fiber diameter measurements that would constitute a statistically reliable sample. In this work a reliable sample is considered to be the number of individual measurements of which three times the probable error of the mean will be equal to or less than a predetermined allowable error. For this work an allowable error of 0.5μ is considered sufficiently small to permit the classification of strains into several classes for the purpose of correlating diameter variations with spinning behavior. In applying diameter measurements to breeding work, two requisites for the size of sample are obvious: (1) The sample must be large enough so that the test can be depended upon to reveal significant differences between strains or varieties, and (2) the size of sample must be reduced to the minimum number that is statistically reliable in order that as many samples as possible may be handled in the time available.

MATERIALS AND METHODS

In choosing material for diameter measurement studies, strains were selected from among those grown in the "advanced strain" test at the experimental field, Scott, Ark. These strains were not artificially hand-pollinated, but they have been rigidly selected, and under the test field conditions it is believed that less than 2 per cent cross-pollination occurs.

[8] BARRITT, N. W. Op. cit. p. 26. (See footnote 4.)

The Rowden strain used in this test was developed at the Arkansas experiment station, the original selection having been made in 1922. In the first year, this strain was grown adjacent to the progeny rows of other strains of Rowden, and seed was selected from this progeny row for the next year's progeny plot. In the years following, the strain was carried on in progeny plots and in larger multiplication blocks. During this period, the strain was adjacent to closely related Rowden strains, and by this procedure of testing in larger plots which were isolated from unrelated strains, the chance of exposure of the strain to cross-fertilization by unrelated pollen was negligible and was materially reduced even with related pollen.

The seed for planting the test rows of New Boykin 1235 and Lightning Express No. 7 was obtained from reliable commercial breeders whose breeding methods and precautions against contaminations are similar to the methods used at the experiment station.

The method of field sampling employed in collecting this material consisted in taking one lock from each of 100 plants in the advanced-strain test rows. By this method the probable effect of cross-pollination is reduced to a negligible factor, so that where the information secured is to be used as a criterion for selection, it seems reasonably safe to assume that we are dealing with self-fertilized and essentially pure-line material. In selecting the material for this test, an effort was made to get representative strains of the extreme length types grown commercially in the State, and, in addition, one strain of medium length.

The long-staple strain selected was Lightning Express No. 7, with a staple length of 39/32 inches. This is a typical fine, long-staple cotton adapted to the river bottom lands of the eastern section of the State.

The medium-staple strain selected was Rowden 3003, with a staple length of 34/32 inches. This particular sample was classed as soft by the stapler. This length is adapted to the upland region of the State, and is also grown to some extent on the bottom lands.

The short-staple strain selected was New Boykin 1235, with a staple length of 28/32 of an inch, and classed as hard. Although the station does not recommend the growing of a cotton of this staple length, a considerable acreage is utilized for producing short-staple varieties, and for this reason it was included in the test.

The field samples, consisting of 100 locks, were thoroughly mixed in the laboratory previous to ginning. After ginning, the fiber was again mixed thoroughly by dividing and lapping the sample, and a final sample was secured by taking a pinch of fibers from 15 places in the sample. This subsample consisting of the 15 fractions was preserved separately for use in making up the pulls from which the diameter measurements were determined.

The diameter-measuring equipment in use in the laboratory consists of a microscope fitted with apochromatic objectives and equipped with a filar micrometer. A euscope is fitted to the microscope in order to lessen the eye strain occasioned by long-continued measurements. The filar micrometer was calibrated by the use of a stage micrometer, and a table converting micrometer readings into microns was used in recording the fiber dimensions.

The fibers to be measured were secured by pulling apart and lapping the subsample, and withdrawing a thin fraction consisting of from

100 to 200 fibers. This fraction was drawn along a glass slide moistened with an aqueous solution of gentian violet, straightened and paralleled by a gentle sliding pressure of the fingers, and covered with a cover glass. While the measurements of the fiber dimensions were being taken, the sample was kept saturated with an aqueous solution of gentian violet. This solution stains the fibers and assists the observer in determining the focal point at which the image is undisturbed, or least disturbed, by diffraction resulting from the curvature of the fiber walls. Measurements were begun at one side of the slide, and the slide was moved across the field of vision in order that measurements could be made on consecutive fibers without duplication of measurements on any one fiber. Two measurements were made on each fiber. The width (W) is the measurement taken at the widest part of the convolution as it appears in flat view. The thickness (T) is the measurement of the edge-on position of the fiber. These two measurements were taken on consecutive twists of the convolution in order to eliminate errors due to differential development along the fiber profile. Consecutive measurements of fiber width and thickness were made along a considerable number of fibers as a preliminary profile test in order to determine the region in which the sample measurements should be taken. These measurements indicated that there is relatively small variation within, roughly, the central half of the fiber length. Consequently in making the measurements, the fiber sample was straightened and the ends of the fibers paralleled as much as possible so that a comparable central region of the fibers would in all cases be used for measurement.

From the two measurements made on the fibers, two other fiber constants were calculated, (1) the mean ribbon width or mean diameter, $\frac{W+T}{2}$, suggested by Barritt,[9] and (2) the dimension ratio, $\frac{W}{T}$ The mean diameter measurement was proposed by Barritt as a means of masking the effect of underdeveloped fibers in the sample so that they might not seriously affect the mean measurement, which is closely equivalent to the diameter of the turgid fiber. The dimension ratio is suggested as a means of bringing out differences in fiber-wall thickness between varieties having a comparable mean diameter, and as a measure of the variation in fiber-wall thickness within the variety. A dimension ratio of 1.0, if it were ever obtained, would mean no collapse of the fiber wall and consequently no convolutions; and, conversely, the larger the ratio the thinner the fiber wall. An extremely high ratio indicates immaturity, and a comparison of the ratios for a number of strains gives a relative measure of the thickness of the fiber walls. Fiber-wall thickness, when considered in connection with the fiber diameter is closely associated with the number of convolutions per unit length, and since the number of convolutions is associated with drag, with the highest warp count for which each cotton is suitable, and with yarn strength, it seems reasonable to believe that additional information of value might be obtained through correlating the dimension ratio of strains with their spinning qualities.

The coefficient of variability of the diameter ratio for a sample reflects the irregularities in fiber-wall thickness that exist in the

[9] Barritt, N. W. Op. cit. (See footnote 4.)

sample, and so should give information of value concerning the uniformity of the fiber-wall development of the sample. Thus it may become valuable as a basis for predicting the probable spinning value of new strains when the necessary correlations of uniformity with certain spinning qualities have been established by spinning tests.

The mean and probable error of the mean for samples of various sizes, and the probable error of the individual derived from 500 measurements for each strain, were calculated by following the machine method of calculation presented by Wallace and Snedecor.[10] This method eliminates errors which might be introduced by grouping the measurements into a frequency distribution. The probable error of the individual derived from 500 measurements or calculated constants was taken as the probable error of the population in the calculations of the size of sample which has statistical significance.

STATISTICAL DATA

FREQUENCY DISTRIBUTIONS

In order to determine whether sufficient skewness was present in any case to invalidate the use of the probable error concept in interpreting the results, the original diameter measurements taken on the fibers and the two calculated constants derived from them were grouped independently for each of the three strains used in the test.

The frequency distributions for the various dimension measurements and calculated constants for the three strains are presented in Table 1. A comparison of the range in classes between the distributions for the three strains brings out the fact that there is a significantly smaller range for the dimension measurements in the case of Lightning Express No. 7 than for Rowden 3003 and New Boykin 1235. This result was to have been expected, inasmuch as Lightning Express No. 7 is a finer cotton than either of the other two. This finding also agrees with previous work in which it was shown that fine cottons exhibit greater uniformity of diameter than coarse ones. The range of distribution for width, thickness, and $\frac{W+T}{2}$ in the case of Rowden 3003 and New Boykin 1235 agrees very closely, indicating that the diameter characteristics for these two strains as measured by these dimension constants are very similar, and that their uniformity as measured on the basis of variation is also similar. However, a somewhat different picture of the two varieties is presented by a comparison of the range of the dimension ratio, $\frac{W}{T}$. A comparison of the two varieties by the use of this constant shows that the range in the case of the Rowden 3003 is nearly 50 per cent greater than of New Boykin 1235. This fact indicates a more uneven development of the fiber wall in the case of Rowden. Theoretically this might be accounted for as due to a possible limitation in fiber nutrient supply occasioned by selection for staple length while at the same time retaining the primary envelope diameter of a short-staple cotton. This variability is of course reflected in the probable error of the mean and the coeffi-

[10] WALLACE, H. A., and SNEDECOR, G. W. CORRELATION AND MACHINE CALCULATION. Iowa State Col. Off. Pub. v. 23, no. 35, 47 p.

cient of variability, and these will be mentioned later in connection with a discussion of the means of the strains.

TABLE 1.—*Frequency distributions for diameter measurements of three strains of cotton*

Class (microns)	Lightning Express No. 7			Rowden 3003			New Boykin 1235			Ratio distribution			
	W	*T*	$\frac{W+T}{2}$	*W*	*T*	$\frac{W+T}{2}$	*W*	*T*	$\frac{W+T}{2}$	Ratios	Lightning Express No. 7	Rowden 3003	New Boykin 1235
7.1–8		4			3								
8.1–9		28			16			1		1.11–1.2		2	
9.1–10		110			29			28		1.21–1.3		8	1
10.1–11		138			53			33		1.31–1.4	7	15	11
11.1–12		63			56			35		1.41–1.5	5	46	42
12.1–13		87	7		93			101		1.51–1.6	29	66	63
13.1–14		64	38		121	8		148	2	1.61–1.7	71	59	114
14.1–15		6	99		55	24		66	14	1.71–1.8	90	63	85
15.1–16	2		124		30	61		42	40	1.81–1.9	68	62	76
16.1–17	2		128		19	75		23	64	1.91–2.0	71	40	46
17.1–18	22		79	6	17	87	2	15	106	2.01–2.1	64	44	25
18.1–19	68		23	27	7	98	11	5	121	2.11–2.2	50	27	11
19.1–20	74		2	32	1	61	19	2	73	2.21–2.3	24	20	9
20.1–21	105			53		41	50	1	36	2.31–2.4	5	14	8
21.1–22	116			55		19	65		24	2.41–2.5	7	12	3
22.1–23	71			85		16	79		10	2.51–2.6	5	3	5
23.1–24	34			76		8	112		8	2.61–2.7	2	8	1
24.1–25	6			52		1	59			2.71–2.8		5	
25.1–26				22		1	31		2	2.81–2.9	1	4	
26.1–27				22			40			2.91–3.0	1		
27.1–28				43			21			3.01–3.1		1	
28.1–29				11			4			3.11–3.2			
29.1–30				7			3			3.21–3.3		1	
30.1–31				2			2			3.21–3.3			
31.1–32				4			2						
32.1–33				2									
33.1–34				1									
34.1–35				1									
35.1–36				1									
36.1–37				1									
Total	500	500	500	500	500	500	500	500	500		500	500	500
Range in classes	10	8	8	20	13	13	15	13	13		17	22	15
Machine method: Mean	20.720	11.073	15.895	23.243	12.906	18.072	23.2862	13.4198	18.355		1.8962	1.8557	1.75588
P. E. of mean	.0469	.0387	.0354	.0819	.0620	.0577	.0663	.0555	.0504		.00662	.0095	.00688
Grouping method: Mean	20.730	11.100	15.884	23.260	12.956	18.072	23.332	13.442	18.332		1.897	1.8404	1.762
P. E. of mean	.0507	.0465	.0414	.0893	.0663	.0631	.0705	.0593	.0582		.00731	.0102	.00696
Difference between methods: Mean	.010	.027	.011	.017	.050	.000	.0458	.0222	.023		.0008	.0153	.00612

The grouping of the measurements and constants for the purpose of constructing frequency graphs also made possible, with little additional calculation, a comparison of the means as calculated by the machine method and by a frequency method. This comparison is valuable for checking the technic used, since it shows whether rounding off in the conversion table has a tendency to cause a skewness in the distribution within the size of class used. The close agreement between the values obtained by grouping and calculating by means of a frequency distribution having class intervals of 1μ and the values secured by the machine method, indicates that neither the rounding

off of the conversion table into fractions of 0.1μ nor the grouping into class intervals of 1μ introduces any cumulative errors which significantly affect the mean of the sample. The difference between the means as calculated by the two methods is recorded in Table 1. The probable error of the mean difference is omitted because comparatively few mean observations are available from which to obtain the correlation coefficient that should enter into the calculation of the probable error of the mean difference. In view of the fact that it is universally agreed that no statistically significant errors are introduced by proper grouping, the small mean differences are interpreted as indicating that the grouping employed is satisfactory.

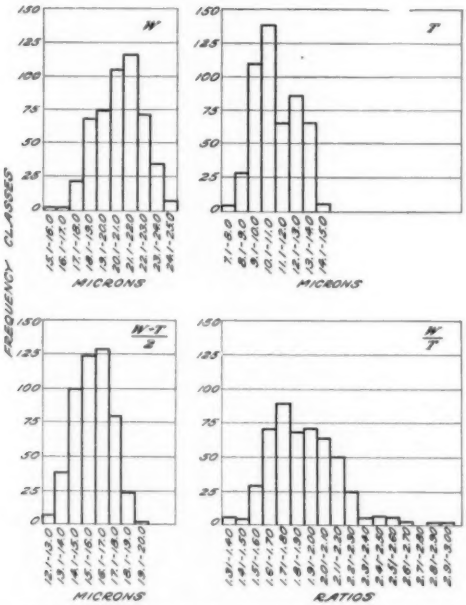

FIGURE 1.—Frequency distribution of diameter measurements of Lightning Express No. 7. W=width; T=thickness; $\frac{W+T}{2}$=mean diameter; $\frac{W}{T}$=diameter dimension ratio. Numerals on abscissa indicate classes employed in grouping the individuals; numerals on ordinate indicate frequency of classes

Figures 1, 2, and 3 present the frequency distribution of the four fiber dimension constants for each of the three strains. There appears to be no constant tendency to multimodality or skewness in any particular direction, when all of the graphs are considered. One class in the thickness graph of Lightning Express No. 7, and one in the width graph of Rowden 3003, have a frequency greater than that expected from their position in the curve, but it is believed that these variations are not sufficiently great to indicate bimodality. In the $\frac{W+T}{2}$ graphs, high classes are smoothed out in each instance,

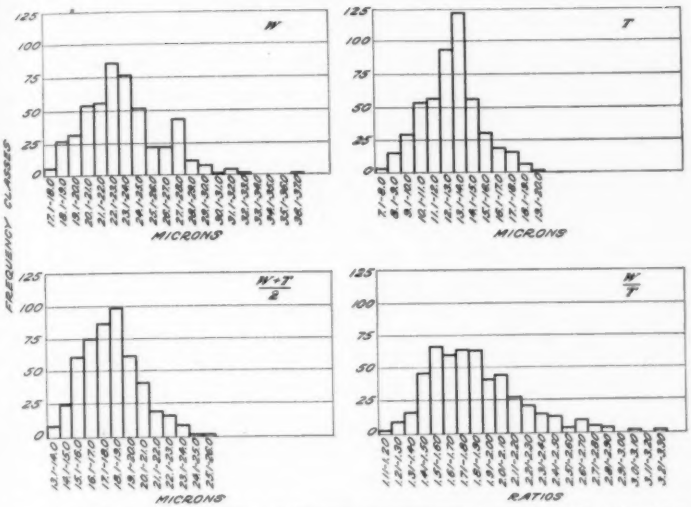

FIGURE 2.—Frequency distribution of diameter measurements of Rowden 3003. W=width; T=thickness; $\frac{W+T}{2}$=mean diameter; $\frac{W}{T}$=diameter dimension ratio. Numerals on abscissa indicate classes employed in grouping the individuals; numerals on ordinate indicate frequency of classes

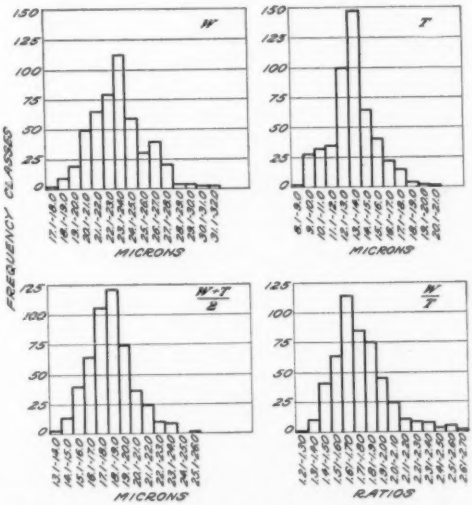

FIGURE 3.—Frequency distribution of diameter measurements of New Boykin 1235. W=width; T=thickness; $\frac{W+T}{2}$=mean diameter; $\frac{W}{T}$=diameter dimension ratio. Numerals on abscissa indicate classes employed in grouping individuals; numerals on ordinate indicate frequency of classes

suggesting that these individual measurement aberrations are due to abnormal fiber-wall development rather than to genetic variation. This averaging of the $\frac{W+T}{2}$ calculation exerts a smoothing out effect on the mean diameter values for abnormally developed fibers, and for this reason it appears to give a better measure of the diameter of the turgid fibers of a sample than either of the two individual measurements from which it was calculated.[11] At the same time, however, it has a tendency to mask abnormalities in cellulose deposit in the fiber walls, as previously mentioned.

The frequency graphs of the $\frac{W}{T}$ ratios exhibit in each case a more extended distribution in the direction of high ratios. These high ratios are the result of a subnormal development of the fiber walls, and thus the deviation from normality might be interpreted as due to the immaturity of the sample rather than to genetic variation. The $\frac{W}{T}$ ratios for Rowden 3003 reach a higher extreme in addition to having a greater range than those for the other two samples. This fact indicates the presence of greater immaturity in the Rowden sample than in either of the others included in this study.

CUMULATIVE MEANS

The mean and probable error of the mean were calulated for successive units of 10 from 10 to 500 observations. These data are given for each of the four dimensional measurements in each of the three strains, and they constitute the actual measurements and derived calculations secured through random distribution in the three strains studied. From these data, Figures 4 to 9 were constructed. These graphs, which are largely self-explanatory, make possible the direct comparison of the actual means for any size of sample with the mean of the entire 500 measurements, and at the same time present the size of the probable error of the mean and the coefficient of variability for each sample size. (Tables 2, 3, and 4.) The deviations of the actual means from the mean of the entire 500 are entirely due to the effects of random sampling. The rather large differences in some cases which are brought out in the graphs emphasize the importance of care in preparing the sample for measurement. Because of the cumulative nature of the graphs, the presence of a few aberrant units of 10, especially in the smaller size samples, tends to spread a considerable influence for some distance along the graph.

CALCULATED AND THEORETICAL PROBABLE ERRORS OF THE MEAN FOR VARIOUS SIZES OF SAMPLE

Tables 5, 6, and 7 present a comparison of the probable error of the mean as calculated from the actual data, and the theoretical probable error of the mean as computed from the probable error of the individual measurement obtained from 500 observations. This comparison is made for sample size in increment steps of 50, from 50 to 500, inclusive, for each of the four fiber dimension constants in the three different strains. The probable error of the individual

[11] BARRITT, N. W. Op. cit. (See footnote 4.)

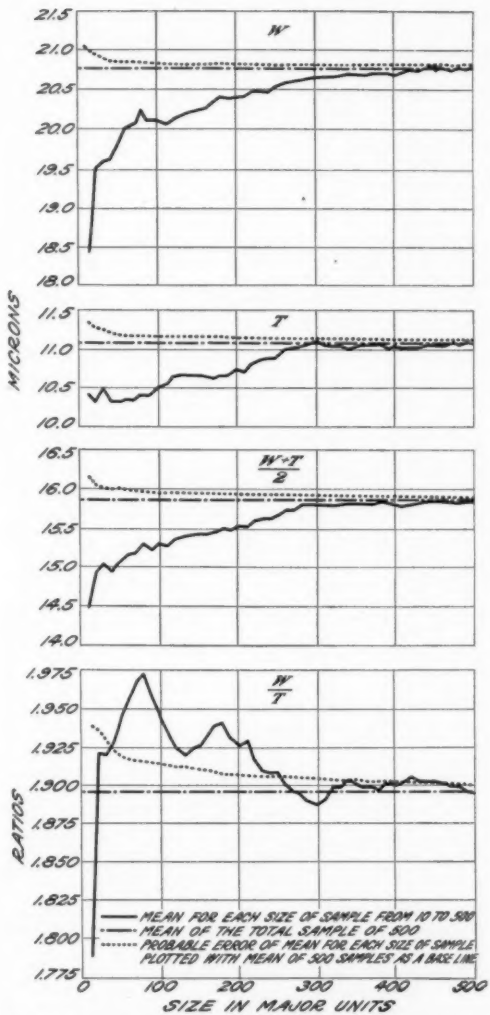
W
T
$\frac{W+T}{2}$
$\frac{W}{T}$
MICRONS
RATIOS
MEAN FOR EACH SIZE OF SAMPLE FROM 10 TO 500
MEAN OF THE TOTAL SAMPLE OF 500
PROBABLE ERROR OF MEAN FOR EACH SIZE OF SAMPLE PLOTTED WITH MEAN OF 500 SAMPLES AS A BASE LINE
SIZE IN MAJOR UNITS

FIGURE 4.—Cumulative mean and probable error of the mean for each of the four diameter measurements in increment steps of 10, from 10 to 500 observations, on Lightning Express No. 7. W=width; T=thickness; $\frac{W+T}{2}$=mean diameter; $\frac{W}{T}$=diameter dimension ratio

recorded in these tables is that calculated from the entire sample of 500 observations, and this value was used in computing the probable error of the different size of sample, by use of the conventional formula for the relation of the probable error of the individual to the probable error of the mean that is, $P.\ E.$ of mean $= \frac{P.E.\text{ of individual}}{\sqrt{n}}$, where n is the size of sample.

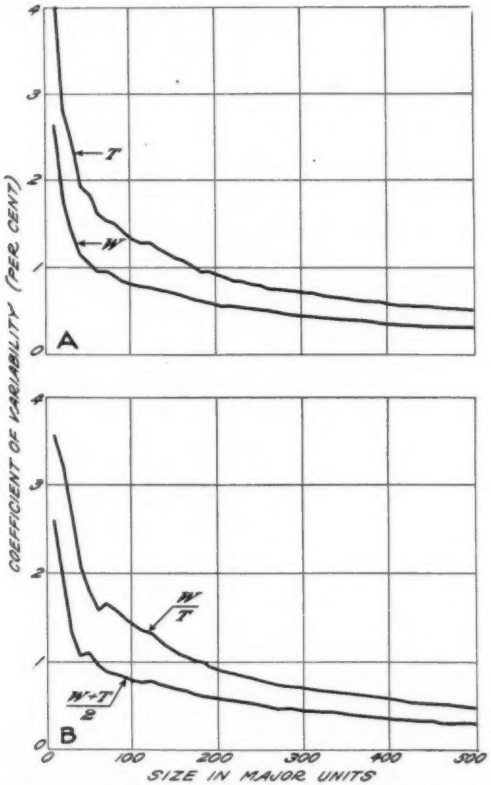

FIGURE 5.—Coefficient of variability for each of the four diameter measurements in increment steps of 10, from 10 to 500 observations, on Lightning Express No. 7. W=width; T=thickness $\frac{W+T}{2}$= mean diameter; $\frac{W}{T}$=diameter dimension ratio. A represents actual measurements made on fibers; B, calculated constants derived from actual measurements

The size of sample which is recorded in these tables as significant for an accuracy of $\pm 0.5\mu$, was calculated by the formula $n = \left(\frac{P.\ E.\text{ individual}}{0.1666667}\right)^2$. This formula was derived from the formula

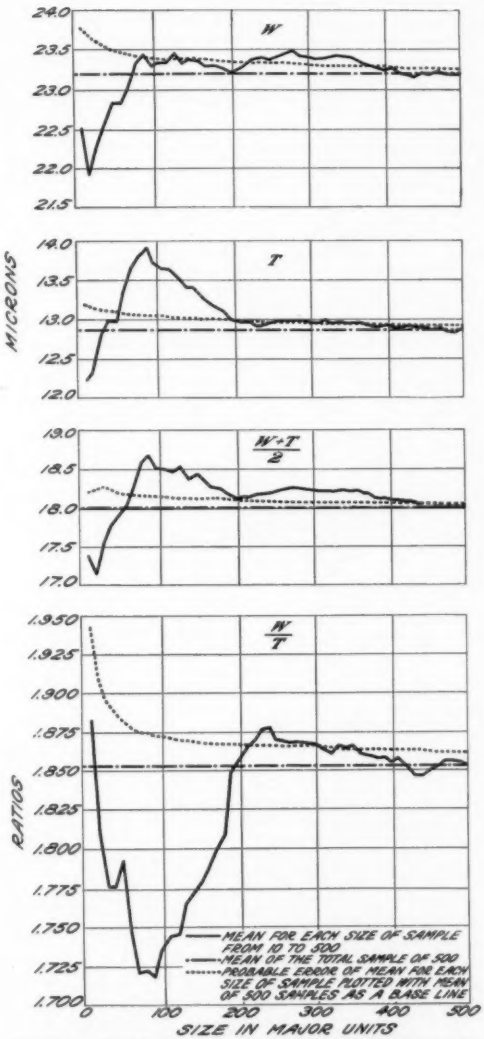
W
T
$\frac{W+T}{2}$
$\frac{W}{T}$
MICRONS
RATIOS
MEAN FOR EACH SIZE OF SAMPLE FROM 10 TO 500
MEAN OF THE TOTAL SAMPLE OF 500
PROBABLE ERROR OF MEAN FOR EACH SIZE OF SAMPLE PLOTTED WITH MEAN OF 500 SAMPLES AS A BASE LINE
SIZE IN MAJOR UNITS

FIGURE 6.—Cumulative mean and probable error of the mean for each of the four diameter measurements in increment steps of 10, from 10 to 500 observations, on Rowden 3003. W=width; T=thickness; $\frac{W+T}{2}$=mean diameter; $\frac{W}{T}$=diameter dimension ratio

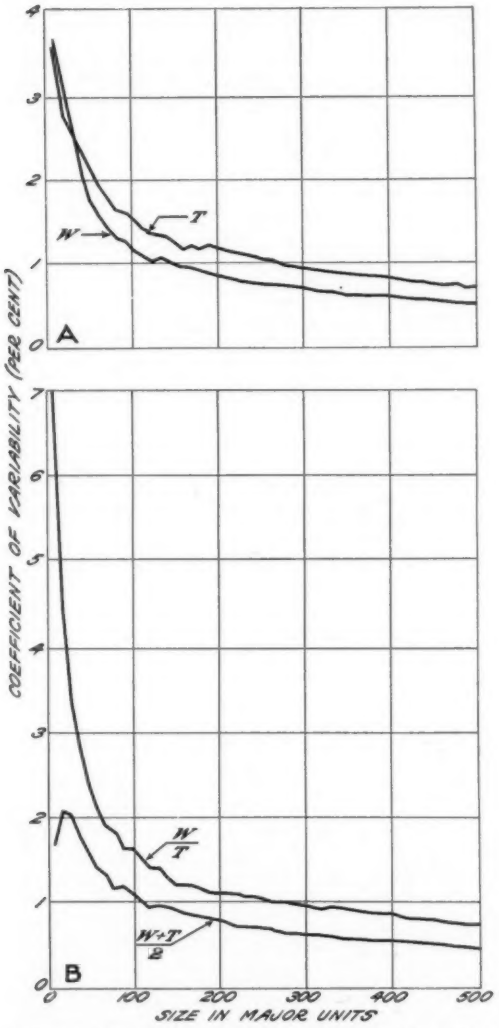

FIGURE 7.—Coefficient of variability for each of the four diameter measurements in increment steps of 10, from 10 to 500 observations, on Rowden 3003. W=width; T=thickness; $\frac{W+T}{2}$=mean diameter; $\frac{W}{T}$=diameter dimension ratio. A represents actual measurements made on fibers; B, calculated constants derived from actual measurements

$0.5 = \frac{3\ P.E.\ \text{of individual}}{\sqrt{n}}$, which expresses the relationship of the probable error of the individual to an empirically selected range, with a consideration of the statistical significance of that range as measured by 3 *P. E.* of the mean.

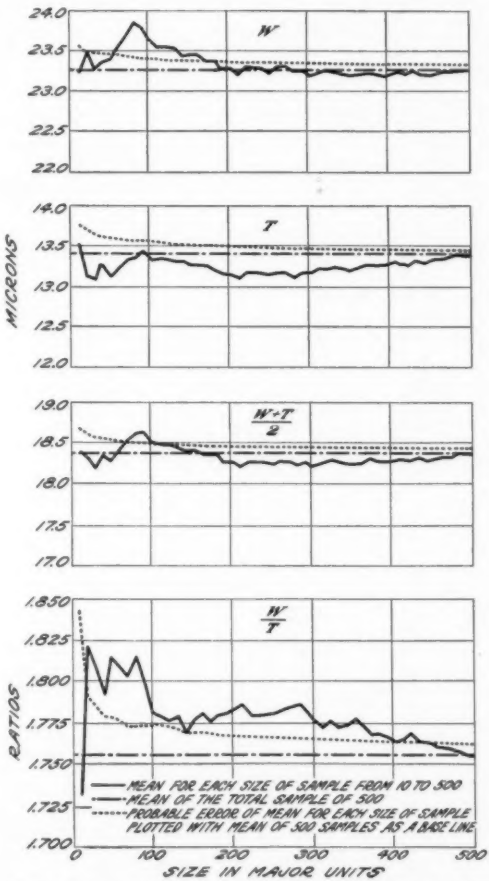
W
T
MICRONS
W+T / 2
W / T
RATIOS
MEAN FOR EACH SIZE OF SAMPLE FROM 10 TO 500
MEAN OF THE TOTAL SAMPLE OF 500
PROBABLE ERROR OF MEAN FOR EACH SIZE OF SAMPLE PLOTTED WITH MEAN OF 500 SAMPLES AS A BASE LINE
SIZE IN MAJOR UNITS

FIGURE 8.—Cumulative mean and probable error of the mean for each of the four diameter measurements in increment steps of 10, from 10 to 500 observations, on New Boykin 1235. W=width; T=thickness; $\frac{W+T}{2}$=mean diameter; $\frac{W}{T}$=diameter dimension ratio

The selection of the range of 0.5μ as the variation allowable for statistical significance is of course empirical, and the range must remain so until sufficient research has been done to establish definitely the relation between spinning behavior and differences of various magnitude in the various diameter dimension measurements. In the

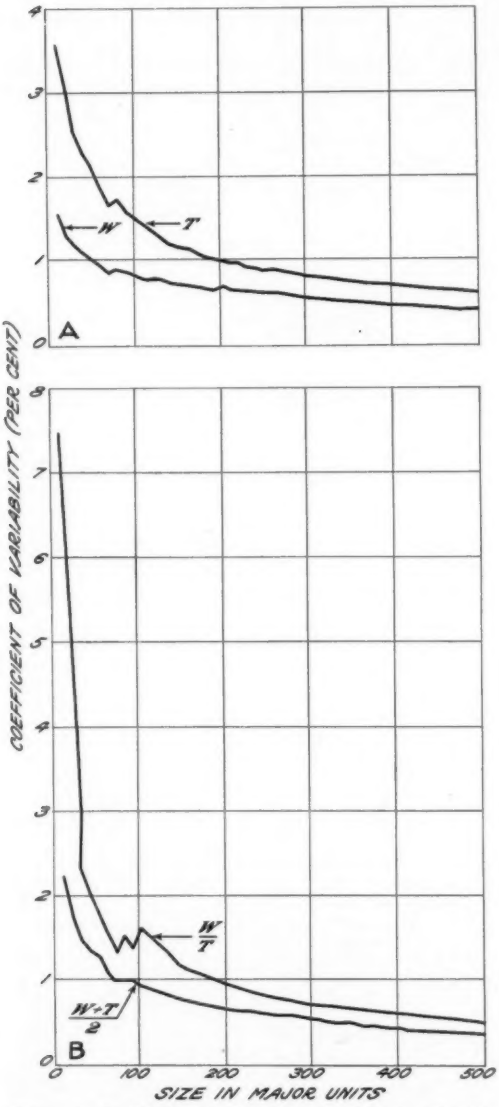

FIGURE 9.—Coefficient of variability for each of the four diameter measurements in increment steps of 10, from 10 to 500 observations, on New Boykin 1235. W=width; T=thickness; $\frac{W+T}{2}$=mean diameter; $\frac{W}{T}$=diameter dimension ratio. A represents actual measurements made on fibers; B, calculated constants derived from actual measurements

three strains studied, the maximum variation is between Lightning Express No. 7 and New Boykin 1235. The difference in width is 2.566μ, in thickness 2.347μ, and in $\frac{W+T}{2}$ 2.460μ. These differences of from 2.3μ to 2.6μ which actually exist between typical commercial types of fine and coarse cottons indicate that a mean derived from a size of sample which is statistically reliable within $\pm 0.5\mu$ will serve at least to divide the strains or varieties into several classes of fineness. After the strains have been selected preliminarily on this basis, spinning tests should indicate the amount of variation in fineness which is significant from the manufacturing standpoint. When these differences have been determined, the breeder will be able to adjust the size of sample necessary to detect variations of such magnitude, and will be able to select his strains on the basis of their predicted manufacturing utility.

TABLE 2.—*The cumulative means ± the probable error and the coefficient of variability for 500 measurements of Lightning Express No. 7 strain of cotton*

Size of sample	W	±P. E.	C. V.	T	±P. E.	C. V.	$\frac{W+T}{2}$	±P. E.	C. V.	$\frac{W}{T}$	±P. E.	C. V.
Number												
10	18.42	0.3292	2.65	10.42	0.2828	4.02	14.47	0.2495	2.56	1.789	0.0435	3.61
20	19.52	.2336	1.77	10.33	.1965	2.82	14.95	.1551	1.54	1.921	.0422	3.26
30	19.61	.1870	1.41	10.48	.1713	2.42	15.06	.1340	1.32	1.904	.0339	2.64
40	19.63	.1534	1.16	10.33	.1349	1.94	14.99	.1087	1.07	1.928	.0269	2.07
50	19.79	.1438	1.08	10.33	.1285	1.85	15.07	.1101	1.08	1.945	.0239	1.82
60	20.01	.1318	.976	10.36	.1128	1.61	15.19	.0985	.961	1.957	.0213	1.61
70	20.06	.1310	.968	10.35	.1080	1.55	15.21	.0909	.886	1.968	.0220	1.66
80	20.21	.1262	.926	10.41	.1054	1.50	15.31	.0889	.861	1.973	.0214	1.61
90	20.08	.1167	.862	10.42	.0992	1.41	15.25	.0835	.812	1.958	.0199	1.51
100	20.10	.1123	.828	10.50	.0941	1.33	15.31	.0792	.767	1.946	.0192	1.46
110	20.06	.1087	.803	10.55	.0919	1.29	15.31	.0775	.751	1.935	.0182	1.39
120	20.12	.1079	.795	10.65	.0919	1.28	15.39	.0789	.769	1.923	.0173	1.34
130	20.15	.1037	.763	10.68	.0883	1.23	15.42	.0764	.735	1.921	.0165	1.27
140	20.20	.0991	.727	10.68	.0840	1.17	15.44	.0735	.705	1.923	.0155	1.19
150	20.22	.0967	.709	10.67	.0802	1.11	15.44	.0716	.687	1.926	.0147	1.13
160	20.25	.0946	.693	10.65	.0778	1.08	15.45	.0701	.672	1.933	.0142	1.09
170	20.31	.0905	.660	10.63	.0736	1.03	15.48	.0664	.636	1.940	.0136	1.04
180	20.39	.0865	.629	10.65	.0707	.983	15.52	.0634	.605	1.942	.0131	1.00
190	20.37	.0841	.612	10.68	.0689	.957	15.52	.0621	.593	1.936	.0126	.964
200	20.37	.0815	.593	10.73	.0670	.926	15.55	.0605	.577	1.927	.0121	.930
210	20.39	.0795	.578	10.73	.0653	.903	15.56	.0594	.566	1.929	.0117	.898
220	20.44	.0778	.564	10.83	.0630	.862	15.63	.0575	.545	1.917	.0113	.764
230	20.44	.0758	.550	10.87	.0621	.847	15.66	.0567	.537	1.909	.0109	.850
240	20.43	.0746	.541	10.88	.0606	.826	15.66	.0556	.626	1.908	.0107	.832
250	20.50	.0722	.522	10.90	.0589	.801	15.70	.0539	.509	1.909	.0104	.804
260	20.54	.0705	.508	10.97	.0576	.779	15.76	.0524	.493	1.902	.0101	.789
270	20.56	.0687	.496	11.01	.0566	.762	15.78	.0513	.482	1.897	.0098	.770
280	20.58	.0669	.482	11.03	.0565	.759	15.81	.0507	.475	1.895	.0097	.762
290	20.61	.0060	.475	11.08	.0554	.742	15.84	.0502	.469	1.890	.0095	.743
300	20.62	.0650	.467	11.09	.0545	.728	15.55	.0495	.462	1.888	.0092	.725
310	20.62	.0635	.457	11.06	.0529	.709	15.84	.0481	.450	1.893	.0090	.707
320	20.64	.0623	.448	11.04	.0517	.695	15.84	.0470	.440	1.899	.0089	.694
330	20.65	.0612	.439	11.04	.0509	.683	15.84	.0462	.433	1.900	.0087	.682
340	20.66	.0600	.431	11.01	.0498	.671	15.84	.0451	.422	1.905	.0087	.679
350	20.65	.0593	.426	11.02	.0490	.659	15.83	.0444	.416	1.903	.0086	.668
360	20.66	.0583	.419	11.04	.0479	.643	15.85	.0437	.408	1.900	.0084	.652
370	20.68	.0574	.411	11.04	.0469	.630	15.86	.0428	.400	1.900	.0082	.641
380	20.68	.0565	.405	11.06	.0460	.617	15.87	.0420	.392	1.898	.0081	.630
390	20.68	.0556	.399	11.03	.0450	.605	15.86	.0412	.385	1.902	.0079	.618
400	20.65	.0544	.390	11.02	.0440	.592	15.84	.0402	.377	1.902	.0078	.606
410	20.67	.0535	.384	11.01	.0435	.585	15.84	.0396	.371	1.904	.0077	.598
420	20.70	.0524	.375	11.01	.0429	.578	15.85	.0389	.364	1.906	.0076	.590
430	20.70	.0513	.367	11.02	.0420	.565	15.86	.0381	.356	1.904	.0074	.578
440	20.71	.0503	.360	11.03	.0415	.557	15.87	.0374	.350	1.904	.0073	.569
450	20.74	.0495	.354	11.04	.0410	.550	15.89	.0369	.345	1.904	.0072	.560
460	20.73	.0490	.351	11.04	.0406	.545	15.89	.0368	.343	1.903	.0071	.550
470	20.72	.0487	.349	11.05	.0403	.540	15.88	.0367	.343	1.901	.0069	.541
480	20.72	.0483	.345	11.05	.0399	.535	15.88	.0364	.340	1.901	.0068	.533
490	20.73	.0474	.339	11.07	.0393	.526	15.90	.0358	.334	1.898	.0067	.525
500	20.72	.0469	.3352	11.07	.0387	.5180	15.90	.0354	.3300	1.896	.0066	.5174
P. E. of individual		1.0487			.8654			.7916			.1476	

Correlation coefficient, $r_{WT}=0.4555$.

TABLE 3.—*The cumulative means ± probable error and the coefficient of variability for 500 measurements of Rowden 3003 strain of cotton*

Size of sample	W	± P. E.	C. V.	T	± P. E.	C. V.	$\frac{W+T}{2}$	± P. E.	C. V.	$\frac{W}{T}$	± P. E.	C. V.
Number												
10	22.544	0.5582	3.671	12.233	0.2979	3.610	17.393	0.1987	1.694	1.8842	0.0890	7.003
20	21.937	.4617	3.120	12.342	.2335	2.805	17.141	.2394	2.071	1.8107	.0555	4.544
30	22.303	.3853	2.561	12.762	.2236	2.598	17.533	.2414	2.041	1.7785	.0404	3.368
40	22.607	.3214	2.108	12.967	.2122	2.426	17.786	.2127	1.773	1.7775	.0344	2.869
50	22.832	.2759	1.792	12.966	.1923	2.199	17.898	.1912	1.584	1.7942	.0294	2.429
60	22.854	.2433	1.578	13.322	.1762	1.961	18.087	.1742	1.428	1.7508	.0250	2.117
70	23.066	.2258	1.451	13.671	.1668	1.809	18.367	.1655	1.336	1.7236	.0222	1.910
80	23.338	.2069	1.314	13.831	.1552	1.664	18.583	.1499	1.196	1.7238	.0213	1.832
90	23.441	.1977	1.250	13.916	.1526	1.626	18.677	.1492	1.184	1.7198	.0193	1.664
100	23.301	.1830	1.164	13.728	.1451	1.567	18.513	.1393	1.116	1.7369	.0192	1.639
110	23.339	.1701	1.081	13.660	.1333	1.447	18.498	.1279	1.025	1.7462	.0180	1.528
120	23.330	.1620	1.029	13.638	.1263	1.373	18.482	.1213	.9730	1.7471	.0170	1.443
130	23.468	.1664	1.051	13.575	.1252	1.367	18.519	.1243	.9951	1.7673	.0167	1.401
140	23.329	.1599	1.016	13.437	.1195	1.319	18.380	.1199	.9671	1.7746	.0158	1.329
150	23.403	.1543	.9775	13.422	.1134	1.253	18.410	.1154	.9293	1.7800	.0149	1.241
160	23.377	.1517	.9621	13.336	.1077	1.197	18.354	.1110	.8966	1.7893	.0148	1.226
170	23.306	.1459	.9282	13.233	.1075	1.204	18.267	.1082	.8782	1.8016	.0148	1.218
180	23.316	.1444	.9182	13.186	.1049	1.179	18.248	.1062	.8628	1.8097	.0144	1.180
190	23.287	.1396	.8889	13.102	.1076	1.218	18.192	.1024	.8345	1.8522	.0140	1.121
200	23.226	.1351	.8624	13.020	.1051	1.197	18.121	.0989	.8092	1.8596	.0142	1.132
210	23.271	.1304	.8308	12.984	.1017	1.161	18.125	.0948	.7754	1.8675	.0141	1.119
220	23.307	.1264	.8040	12.980	.0999	1.141	18.141	.0920	.7519	1.8706	.0141	1.118
230	23.387	.1257	.7969	12.956	.0972	1.112	18.169	.0914	.7458	1.8781	.0137	1.081
240	23.408	.1243	.7873	12.957	.0956	1.094	18.181	.0895	.7298	1.8789	.0136	1.073
250	23.397	.1232	.7807	12.980	.0930	1.062	18.187	.0882	.7190	1.8726	.0133	1.053
260	23.426	.1172	.7544	13.006	.0906	1.033	18.215	.0852	.6935	1.8702	.0128	1.015
270	23.467	.1171	.7398	13.025	.0882	1.004	18.249	.0832	.6759	1.8695	.0130	1.031
280	23.493	.1167	.7365	13.009	.0855	.9744	18.250	.0821	.6670	1.8708	.0127	1.006
290	23.461	.1133	.7160	13.002	.0841	.9590	18.230	.0803	.6531	1.8686	.0124	.9838
300	23.438	.1110	.7021	12.988	.0832	.9497	18.211	.0786	.6399	1.8690	.0123	.9757
310	23.416	.1083	.6857	12.994	.0812	.9265	18.203	.0764	.6223	1.8654	.0121	.9617
320	23.415	.1074	.6800	13.008	.0801	.9129	18.210	.0755	.6147	1.8627	.0119	.9472
330	23.428	.1059	.6702	12.993	.0791	.9026	18.209	.0739	.6017	1.8672	.0122	.9687
340	23.449	.1046	.6613	13.000	.0780	.8895	18.223	.0732	.5955	1.8669	.0120	.9530
350	23.443	.1019	.6444	12.986	.0770	.8791	18.213	.0714	.5812	1.8684	.0118	.9363
360	23.428	.0998	.6316	12.995	.0756	.8625	18.214	.0703	.5722	1.8633	.0115	.9150
370	23.368	.0989	.6275	12.982	.0748	.8542	18.173	.0700	.5711	1.8610	.0113	.9002
380	23.316	.0973	.6187	12.943	.0733	.8396	18.133	.0687	.5617	1.8601	.0112	.8927
390	23.295	.0965	.6142	12.941	.0730	.8363	18.116	.0686	.5614	1.8603	.0111	.8846
400	23.281	.0948	.6037	12.947	.0715	.8188	18.112	.0671	.5493	1.8574	.0109	.8700
410	23.290	.0937	.5965	12.928	.0705	.8085	18.107	.0663	.5429	1.8604	.0107	.8527
420	23.233	.0918	.5858	12.926	.0694	.7960	18.078	.0652	.5347	1.8553	.0105	.8391
430	23.190	.0902	.5764	12.946	.0682	.7810	18.070	.0640	.5251	1.8492	.0103	.8258
440	23.173	.0889	.5688	12.928	.0673	.7718	18.047	.0632	.5191	1.8490	.0102	.8179
450	23.233	.0881	.5622	12.931	.0659	.7556	18.080	.0622	.5100	1.8522	.0100	.8004
460	23.213	.0866	.5530	12.899	.0650	.7471	18.055	.0612	.5025	1.8552	.0099	.7912
470	23.256	.0855	.5451	12.905	.0639	.7341	18.084	.0601	.4927	1.8577	.0098	.7821
480	23.236	.0842	.5372	12.877	.0627	.7219	18.055	.0591	.4853	1.8589	.0097	.7736
490	23.215	.0831	.5307	12.875	.0624	.7185	18.048	.0584	.4797	1.8586	.0096	.7658
500	23.243	.0819	.5224	12.905	.0620	.7123	18.072	.0577	.4734	1.8557	.0095	.7590
P. E. of individual	------	1.8313	------	------	1.3864	------	------	1.2902	------	------	.2124	------

Correlation coefficient $r_{WT}=0.3747$.

The size of sample which is statistically signficant within the range of $\pm 0.5\mu$ varies considerably with the different constants in the different strains, taking values in the increasing order of 23, 27, 40, 52, 55, 60, 69, 79, and 121. The only measurement for which a sample size in excess of 100 was indicated is the width of Rowden 3003. The values for $\frac{W+T}{2}$ are 23, 52, and 60. Considering this measurement as being a more reliable estimate of fineness than either fiber width or fiber thickness alone, in accord with the opinion of Barritt and others, it appears that a sample of 100 fibers measured for width and thickness is large enough to be used in a preliminary study of fineness. Consequent findings will shed additional light on the significant size of sample, and it may be that a larger sample will in some cases be necessary, or that a smaller sample will suffice. In

the three strains investigated, it appears that a sample of the same size will have a greater significance in fine cottons than in coarse ones, because of the greater uniformity of fine cotton. Thus it might be advantageous in case a very detailed analysis of samples was desired to adjust the size of sample for the more extensive investigation on the basis of the variation and fineness of the preliminary sample tested. The average value for size of sample in all of the nine constants is 58.4, and the average for $\frac{W+T}{2}$ is 45. If 100 is selected as the size of sample to be used, we will therefore have a margin of 71.2 per cent over the average for statistical significance. On the same basis, when only the $\frac{W+T}{2}$ average is considered, we have a margin of 122.2 per cent in the size of the sample used.

TABLE 4.—*The cumulative means ± probable error and the coefficient of variability for 500 measurements of New Boykin strain of cotton*

Size of sample	W	± P. E.	C. V.	T	± P. E.	C. V.	$\frac{W+T}{2}$	± P. E.	C. V.	$\frac{W}{T}$	± P. E.	C. V.
Number												
10	23. 22	0. 2426	1. 55	13. 51	0. 3268	3. 59	18. 36	0. 2762	2. 23	1. 732	0. 0872	7. 48
20	23. 47	. 2024	1. 28	13. 11	. 2805	3. 17	18. 29	. 2162	1. 75	1. 822	. 0360	2. 93
30	23. 26	. 1816	1. 16	13. 08	. 2241	2. 54	18. 17	. 1799	1. 47	1. 808	. 0288	2. 36
40	23. 36	. 1711	1. 09	13. 25	. 2036	2. 28	18. 30	. 1663	1. 35	1. 793	. 0244	2. 02
50	23. 39	. 1597	1. 01	13. 12	. 1898	2. 14	18. 26	. 1583	1. 29	1. 815	. 0220	1. 80
60	23. 52	. 1484	. 936	13. 21	. 1662	1. 86	18. 37	. 1339	1. 13	1. 809	. 0196	1. 61
70	23. 69	. 1370	. 857	13. 33	. 1482	1. 65	18. 51	. 1272	1. 02	1. 804	. 0172	1. 41
80	23. 83	. 1444	. 898	13. 37	. 1554	1. 72	18. 60	. 1330	1. 06	1. 814	. 0186	1. 52
90	23. 78	. 1409	. 879	13. 43	. 1466	1. 62	18. 61	. 1287	1. 03	1. 802	. 0169	1. 39
100	23. 63	. 1317	. 826	13. 35	. 1363	1. 51	18. 49	. 1190	. 954	1. 781	. 0195	1. 62
110	23. 55	. 1255	. 790	13. 34	. 1286	1. 43	18. 45	. 1126	. 905	1. 778	. 0182	1. 52
120	23. 56	. 1271	. 800	13. 34	. 1212	1. 35	18. 45	. 1101	. 885	1. 777	. 0169	1. 41
130	23. 54	. 1236	. 779	13. 32	. 1150	1. 28	18. 43	. 1062	. 854	1. 778	. 0158	1. 32
140	23. 46	. 1192	. 754	13. 33	. 1081	1. 20	18. 39	. 1015	. 818	1. 770	. 0147	1. 23
150	23. 47	. 1152	. 728	13. 29	. 1038	1. 16	18. 38	. 0980	. 791	1. 777	. 0140	1. 16
160	23. 46	. 1127	. 713	13. 27	. 1025	1. 15	18. 36	. 0964	. 778	1. 781	. 0135	1. 13
170	23. 39	. 1104	. 700	13. 27	. 0984	1. 10	18. 33	. 0934	. 755	1. 776	. 0129	1. 08
180	23. 38	. 1089	. 691	13. 24	. 0937	1. 05	18. 31	. 0898	. 727	1. 779	. 0126	1. 05
190	23. 29	. 1073	. 683	13. 17	. 0909	1. 02	18. 23	. 0879	. 715	1. 782	. 0123	1. 02
200	23. 29	. 1086	. 692	13. 16	. 0893	1. 01	18. 22	. 0883	. 718	1. 784	. 0118	. 984
210	23. 25	. 1053	. 671	13. 12	. 0862	. 975	18. 19	. 0851	. 693	1. 786	. 0116	. 961
220	23. 30	. 1064	. 677	13. 19	. 0869	. 977	18. 24	. 0869	. 706	1. 781	. 0111	. 924
230	23. 29	. 1043	. 664	13. 19	. 0840	. 944	18. 24	. 0845	. 687	1. 780	. 0108	. 896
240	23. 29	. 1012	. 644	13. 18	. 0817	. 920	18. 23	. 0821	. 667	1. 781	. 0104	. 869
250	23. 27	. 0982	. 625	13. 17	. 0797	. 897	18. 22	. 0794	. 646	1. 782	. 0102	. 851
260	23. 31	. 0995	. 633	13. 19	. 0802	. 901	18. 25	. 0811	. 658	1. 783	. 0100	. 830
270	23. 32	. 0976	. 621	13. 19	. 0797	. 896	18. 25	. 0801	. 651	1. 785	. 0098	. 816
280	23. 27	. 0945	. 602	13. 15	. 0777	. 876	18. 21	. 0777	. 633	1. 787	. 0096	. 799
290	23. 27	. 0925	. 589	13. 18	. 0761	. 856	18. 22	. 0759	. 618	1. 783	. 0094	. 781
300	23. 21	. 0900	. 575	13. 19	. 0744	. 837	18. 20	. 0739	. 602	1. 777	. 0092	. 766
310	23. 24	. 0877	. 560	13. 23	. 0734	. 822	18. 24	. 0723	. 588	1. 774	. 0091	. 759
320	23. 27	. 0864	. 550	13. 24	. 0724	. 810	18. 25	. 0710	. 577	1. 776	. 0090	. 748
330	23. 26	. 0840	. 535	13. 25	. 0711	. 796	18. 25	. 0693	. 563	1. 774	. 0088	. 738
340	23. 23	. 0833	. 531	13. 24	. 0701	. 785	18. 24	. 0684	. 556	1. 774	. 0088	. 734
350	23. 21	. 0817	. 522	13. 21	. 0689	. 773	18. 21	. 0671	. 546	1. 777	. 0087	. 723
360	23. 23	. 0806	. 514	13. 25	. 0678	. 758	18. 24	. 0660	. 536	1. 774	. 0085	. 711
370	23. 24	. 0790	. 504	13. 29	. 0664	. 741	18. 27	. 0646	. 524	1. 769	. 0083	. 698
380	23. 24	. 0776	. 495	13. 29	. 0653	. 728	18. 26	. 0635	. 515	1. 769	. 0082	. 684
390	23. 22	. 0766	. 489	13. 29	. 0643	. 717	18. 25	. 0625	. 508	1. 768	. 0080	. 675
400	23. 23	. 0757	. 484	13. 31	. 0633	. 705	18. 27	. 0617	. 501	1. 765	. 0079	. 663
410	23. 25	. 0745	. 475	13. 31	. 0622	. 693	18. 28	. 0607	. 492	1. 766	. 0078	. 651
420	23. 24	. 0737	. 470	13. 29	. 0618	. 689	18. 27	. 0602	. 488	1. 769	. 0077	. 647
430	23. 26	. 0731	. 466	13. 33	. 0612	. 681	18. 30	. 0598	. 485	1. 765	. 0076	. 636
440	23. 23	. 0718	. 459	13. 33	. 0600	. 668	18. 28	. 0587	. 476	1. 764	. 0074	. 623
450	23. 22	. 0709	. 452	13. 34	. 0592	. 658	18. 28	. 0579	. 470	1. 762	. 0073	. 615
460	23. 24	. 0698	. 445	13. 36	. 0588	. 653	18. 30	. 0572	. 463	1. 761	. 0073	. 613
470	23. 26	. 0693	. 442	13. 38	. 0581	. 643	18. 32	. 0566	. 458	1. 760	. 0072	. 606
480	23. 27	. 0681	. 434	13. 40	. 0573	. 634	18. 34	. 0557	. 450	1. 758	. 0071	. 597
490	23. 28	. 0671	. 427	13. 41	. 0563	. 622	18. 35	. 0547	. 442	1. 756	. 0070	. 588
500	23. 2862	. 0663	. 4221	13. 4198	. 0555	. 613	18. 355	. 0540	. 4363	1. 75588	. 00688	. 5809
P. E. of individual		1. 4825			1. 2410			1. 2075			. 1538	

Correlation coefficient $r_{WT}=0.6553$.

TABLE 5.—*Calculated and theoretical probable errors for the mean of various sizes of sample for Lightning Express No. 7 strain of cotton* [a]

Size of sample	Width		Thickness		$\frac{W+T}{2}$		$\frac{W}{T}$	
	Calculated	Theoretical	Calculated	Theoretical	Calculated	Theoretical	Calculated	Theoretical
Number								
50	0.1438	0.1483	0.1285	0.1224	0.1101	0.1119	0.0239	0.0209
100	.1123	.1049	.0941	.0865	.0792	.0792	.0192	.0148
150	.0967	.0856	.0802	.0707	.0716	.0646	.0147	.0120
200	.0815	.0742	.0670	.0612	.0605	.0560	.0121	.0104
250	.0722	.0663	.0589	.0547	.0539	.0501	.0104	.0093
300	.0650	.0605	.0545	.0500	.0495	.0457	.0092	.0085
350	.0593	.0561	.0490	.0463	.0444	.0423	.0086	.0079
400	.0544	.0524	.0440	.0433	.0402	.0396	.0078	.0074
450	.0495	.0494	.0410	.0408	.0369	.0373	.0072	.0070
500	.0469	.0469	.0387	.0387	.0354	.0354	.0066	.0066
Mean	20.720		11.073		15.895		1.896	
P. E. of individual	1.04872		.865358		.791568		.147580	

[a] Size of sample which would probably indicate a significant mean difference of: 0.5 micron = 40 for width, 27 for thickness, 23 for $\frac{W+T}{2}$; 2.5 per cent of mean = 37 for width, 88 for thickness, 36 for $\frac{W+T}{2}$, and 87 for $\frac{W}{T}$.

TABLE 6.—*Calculated and theoretical probable errors for the mean of various sizes of sample for Rowden 3003 strain of cotton* [a]

Size of sample	Width		Thickness		$\frac{W+T}{2}$		$\frac{W}{T}$	
	Calculated	Theoretical	Calculated	Theoretical	Calculated	Theoretical	Calculated	Theoretical
Number								
50	0.2759	0.2590	0.1923	0.1960	0.1912	0.1825	0.0294	0.0300
100	.1830	.1831	.1451	.1386	.1392	.1290	.0192	.0212
150	.1543	.1495	.1134	.1132	.1154	.1053	.0149	.0173
200	.1351	.1295	.1051	.0980	.0989	.0912	.0142	.0150
250	.1232	.1158	.0930	.0877	.0882	.0816	.0133	.0134
300	.1110	.1057	.0832	.0800	.0796	.0745	.0123	.0123
350	.1019	.0979	.0770	.0741	.0714	.0690	.0118	.0114
400	.0948	.0916	.0715	.0693	.0671	.0645	.0109	.0106
450	.0881	.0863	.0659	.0654	.0622	.0608	.0100	.0100
500	.0819	.0819	.0620	.0620	.0577	.0577	.0095	.0095
Mean	23.243		12.905		18.072		1.856	
P. E. of individual	1.83134		1.38636		1.29021		.212426	

[a] Size of sample which would probably indicate a significant mean difference of: 0.5 micron = 121 for width, 69 for thickness, 60 for $\frac{W+T}{2}$; 2.5 per cent of mean = 89 for width, 166 for thickness, 73 for $\frac{W+T}{2}$ and 189 for $\frac{W}{T}$.

In selecting the range of allowable error for determining differences in mean measurements between strains it might be advantageous, if it were possible in the practical application of this method to breeding operations, to follow the customary statistical procedure of varying the size of the difference to correspond to the varying size of the mean. The difference customarily used is 2.5 per cent of the mean. Unfortunately this method does not appear practical since we have no way of knowing in advance the characteristic mean dimensions of strains which it is desirable to investigate, and for this reason it is believed that, in order to obviate the use of burdensome and impractical determinations on large numbers, it will be necessary to employ a

standard difference for use in the preliminary tests of all strains. In cases where a very detailed study is desired it will of course be better to use the customary statistical difference of 2.5 per cent of the mean.

The size of sample which would probably indicate a significant difference of 2.5 per cent of the mean was calculated for each of the dimensional constants in the three strains reported in this study. (Tables 5, 6, and 7.) These calculations were made by the following adaptation of the conventional formula which expresses the relationship of the probable error of the individual to the probable error of the mean: $N=\left(\frac{P.E.\text{ individual}}{\text{Mean}\times 0.008333}\right)^2$. It is apparent that there is considerable variation in the size of sample when considered on the basis of 2.5 per cent of the mean and on the basis of the standard difference of 0.5μ. This standard difference of 0.5μ is equivalent to 2.5 per cent of a mean of 20.0μ, and it is therefore to be expected that when the mean diameter is actually greater than 20.0μ the size of sample that may be considered significant will be reduced, and vice versa. These expected changes in significant sample size are apparent in the data presented. The greatest of these changes are found in the size of sample for thickness. Since the mean thickness is considerably below 20.0μ, we get a marked increase in sample size, and in the case of Rowden and New Boykin the sample size indicated exceeds the 100 measurements which the use of a 0.5μ difference indicated as being sufficiently large to show a significant mean difference. However, the indicated number for sample size in the $\frac{W+T}{2}$ measurements ranges from 23 to 73, and since this appears to be preferable to either W or T alone for comparing strains, it is concluded that the sample size of 100 is, in all the cases considered, sufficiently large to indicate statistically significant differences between samples, whether considered on the basis of an empirical 0.5μ range or on the basis of 2.5 per cent of the mean.

TABLE 7.—*Calculated and theoretical probable error for the mean of various size of sample for New Boykin 1235 strain of cotton* [a]

Size of sample	Width		Thickness		$\frac{W+T}{2}$		$\frac{W}{T}$	
	Calculated	Theoretical	Calculated	Theoretical	Calculated	Theoretical	Calculated	Theoretical
Number								
50	0.1597	0.2097	0.1898	0.1755	0.1583	0.1708	0.0220	0.0218
100	.1317	.1483	.1363	.1241	.1190	.1290	.0195	.0154
150	.1152	.1210	.1038	.1013	.0980	.0986	.0140	.0126
200	.1086	.1048	.0893	.0878	.0883	.0854	.0118	.0109
250	.0982	.0938	.0797	.0785	.0794	.0764	.0102	.0097
300	.0900	.0856	.0744	.0717	.0739	.0697	.0092	.0089
350	.0817	.0792	.0689	.0663	.0671	.0645	.0087	.0082
400	.0757	.0741	.0633	.0621	.0617	.0604	.0079	.0077
450	.0709	.0699	.0592	.0585	.0579	.0569	.0073	.0073
500	.0663	.0663	.0555	.0555	.0540	.0540	.0069	.0069
Mean	23.286		13.420		18.355		1.756	
P. E. of individual	1.48251		1.24102		1.20748		.153841	

[a] Size of sample which would probably indicate a significant mean difference of: 0.5 microns = 79 for width, 55 for thickness, 52 for $\frac{W+T}{2}$; 2.5 per cent of mean = 58 for width, 123 for thickness, 62 for $\frac{W+T}{2}$ and 111 for $\frac{W}{T}$.

The size of sample was calculated for the $\frac{W}{T}$ ratio on the 2.5 per cent of the mean basis. In Lightning Express No. 7 the number was found to be 87; in New Boykin 1235, 111; and in Rowden 3003, 189. In the case of Rowden 3003 the number indicated is considerably in excess of the sample size of 100 which has been considered sufficient for the other measurements. This ratio is designed to reveal irregularity in the development of the fiber walls in a sample, and when an unusual amount of irregularity exists it will be reflected in the size of the probable error, which in turn will affect the size of sample. The frequency distribution of $\frac{W}{T}$ for Rowden 3003 (Table 1) indicates that it is the most variable of the three strains. The coefficient of variation (fig. 7) also shows greater variability of the diameter ratio. In view of these facts it is not believed that the indicated sample size of 189 in this case invalidates the use of 100 as a sufficient number.

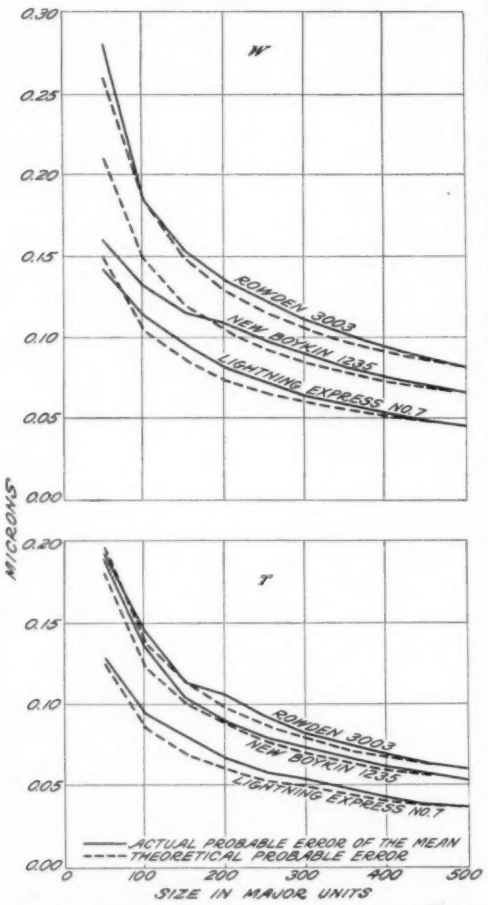

FIGURE 10.—Actual probable error of the mean for various sizes of sample, in increment steps of 50, from 50 to 500 observations, as compared with the theoretical probable error of the mean calculated from the probable error of the individual for the entire sample. The three strains are compared for each diameter constant. W= width; T= thickness

Figures 10 and 11 present in graphic form the data contained in Tables 5, 6, and 7. Generally speaking, it is noticeable that the curves showing the size of the theoretical probable errors for the cumulative means are in rather good agreement with those representing the actual probable errors determined from the original data. There appears to be a slight tendency for the actual values to exceed the theoretical

in the majority of cases. However, in more than half the curves, it is found that at some point the actual values drop below the theoretical. The greatest discrepancy found in any case is in the 50 sample for width in New Boykin 1235, where the theoretical exceeds the actual by 0.05μ. The significance of this difference was tested by the application of the conventional formula for the probable error of a probable error, $0.6745 \frac{P.E.}{\sqrt{2n}}$. Substituting the values in this case we have, $0.6745 \frac{0.2097}{\sqrt{100}}$, which equals 0.014. Therefore this greatest difference might easily be attributed to errors of random sampling. In the other curves, the difference does not exceed 0.01μ for any of the sample sizes tested. The fact that the theoretical and actual curves always coincide at 500 is due to the fact that the probable error of the individual as determined from 500 observations was used for calculating the theoretical curves.

A comparison of the theoretical sample sizes with those showing the corresponding calculated probable error values in Tables 2, 3, and 4 reveals a close relationship in the majority of cases. These data were assembled for convenience in making comparisons between the actual and theoretical. (Table 8.) The actual size of sample is recorded as the two classes between which the probable error corresponding to the theoretical number lies, in all cases except one in which the probable error corresponded exactly to one class. In the

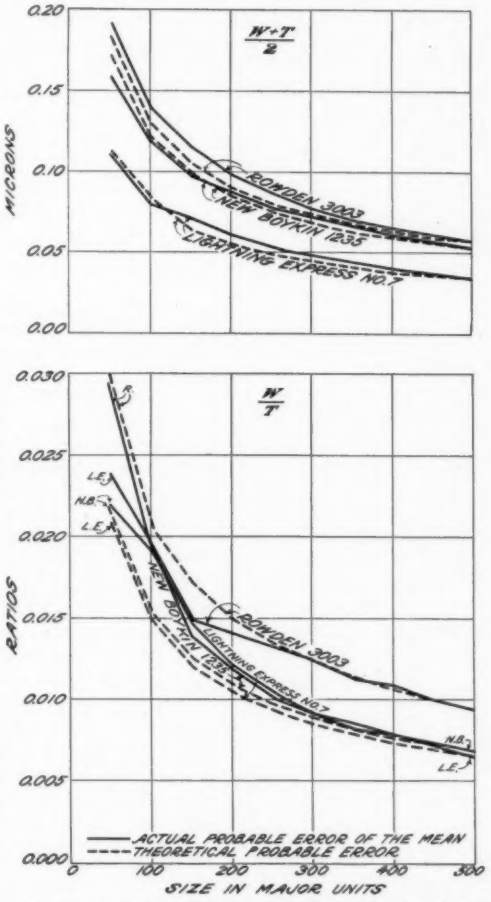

FIGURE 11.—Actual probable error of the mean for various sizes of sample, in increment steps of 50, from 50 to 500 observations, as compared with the theoretical probable error of the mean calculated from the probable error of the individual for the entire sample. The three strains are compared for each diameter constant.

$\frac{W+T}{2}$ = mean diameter; $\frac{W}{T}$ = diameter dimension ratio

comparison on the basis of a 0.5 μ range for significant differences, the only instance in which a marked deviation from the theoretical is found is in the W determination for New Boykin 1235. In this case the actual is considerably less than the theoretical, indicating the existence of a favorable random assortment in the original data. The comparison on the basis of 2.5 per cent of the mean shows a close fit in all cases except the W constant for New Boykin 1235, the T constant for Lightning Express No. 7, and the $\frac{W}{T}$ constant for all of the strains. The actual number for the W of New Boykin 1235 is only about half of the theoretical, indicating the influence of favorable random sampling as in the parallel case where a significant range of 0.5 μ was used for the same constant in the same strain. The actual sample size in Lightning Express No. 7 is somewhat larger than the theoretical, indicating the influence of slightly unfavorable random sampling.

Since the $\frac{W}{T}$ ratio is designed to uncover irregularities in fiber-wall development between strains it is not surprising to find larger variations in this constant than in the other measurements. The variations between the actual and theoretical numbers are somewhat larger than for the other constants. In two cases the actual number exceeds the theoretical and in one case the theoretical exceeds the actual. It is believed that these differences, in view of the fact that the constant is designed to show irregularities in development, may be safely considered as due to errors of random sampling.

TABLE 8.—*A comparison of theoretical sample sizes with those showing the corresponding calculated probable error in Tables 2, 3, and 4, as determined for a range of 0.5 micron, and of 2.5 per cent of the mean for three strains of cotton*

Value	Range for significant differences	Lightning Express No. 7			
		W	T	$\frac{W+T}{2}$	$\frac{W}{T}$
Theoretical	0.5 micron	40	27	23	
Actual	do	30–40	30–40	10–20	
Theoretical	2.5 per cent of mean	37	88	36	87
Actual	do	30–40	100–110	30–40	130–140

Value	Range for significant differences	Rowden 3003				New Boykin 1235			
		W	T	$\frac{W+T}{2}$	$\frac{W}{T}$	W	T	$\frac{W+T}{2}$	$\frac{W}{T}$
Theoretical	0.5 micron	121	69	60		79	55	53	
Actual	do	110–120	70	60–70		40–50	50–60	40	
Theoretical	2.5 per cent of mean	89	166	73	189	58	123	62	111
Actual	do	90–100	170	70–80	140–150	20–30	130–140	50–60	140–150

TABLE 9.—*Comparison of mean diameter measurements for three strains of cotton, on a basis of 500 measurements*

Strain	Width			Thickness		
	Mean	±P. E.	C. V.	Mean	±P. E.	C. V.
			Per cent			*Per cent*
Lightning Express No. 7	20. 720	0. 0469	0. 3356	11. 073	0. 0387	0. 5181
Rowden 3003	23. 243	. 0819	. 5224	12. 905	. 0620	. 7123
New Boykin 1235	23. 286	. 0663	. 4221	13. 420	. 0555	. 6130

Strain	$\frac{W+T}{2}$			$\frac{W}{T}$			Correlation r_{WT}
	Mean	±P. E.	C. V.	Mean	±P. E.	C. V.	
			Per cent			*Per cent*	
Lightning Express No. 7	15. 895	0. 0354	0. 3302	1. 896	0. 00662	0. 5174	0. 4555
Rowden 3003	18. 072	. 0577	. 4734	1. 856	. 0095	. 7590	. 3747
New Boykin 1235	18. 355	. 0504	. 4071	1. 756	. 00688	. 5800	. 6553

COMPARISON OF DIAMETER MEASUREMENTS FOR THE THREE STRAINS

Table 9 presents the mean, the probable error of the mean, and the coefficient of variability for the four diameter measurements for each of the three strains, together with the coefficient of correlation for width and thickness in each strain. The correlation coefficients for the dimensional measurements other than width and thickness were not calculated, because width or thickness, or both, would enter into the values correlated more than once. The rank of the three strains with regard to the value of r for width and thickness is: New Boykin 1235, 0.6553; Lightning Express No. 7, 0.4555; and Rowden 3003, 0.3747. The high correlation between width and thickness in New Boykin 1235 indicates that this is the most uniform of the three strains in respect to thickness of fiber wall. This strain has the shortest staple length of the three strains tested. Lightning Express No. 7, which has the intermediate correlation coefficient, has the longest staple and the smallest diameter measurements, and Rowden 3003, which has the lowest correlation coefficient, has diameter measurements closely approaching New Boykin, with a staple length intermediate between New Boykin and Lightning Express. It would be interesting to know whether this low correlation coefficient points to an uneven deposition of cellulose on the fiber walls, occasioned by a lack of physiological balance resulting from selection for staple length while retaining the large diameter of a coarse short-staple cotton.

TABLE 10.—*Mean difference of diameter measurements for three strains of cotton on a basis of 500 measurements*

Strains	Width				Thickness			
	Mean difference	σMD	$\frac{MD}{\sigma MD}$	Odds	Mean difference	σMD	$\frac{MD}{\sigma MD}$	Odds
Lightning Express No. 7 and Rowden 3003.	2.523	0.13986	18.039	Infinite	1.832	0.10835	16.908	Infinite.
Lightning Express No. 7 and New Boykin 1235.	2.566	.12036	21.319	do	2.347	.10030	23.400	Do.
Rowden 3003 and New Boykin 1235.	0.043	.15622	0.2753	1:.353	0.515	.12337	4.174	32,894:1.

Strains	$\frac{W+T}{2}$				$\frac{W}{T}$			
	Mean difference	σMD	$\frac{MD}{\sigma MD}$	Odds	Mean difference	σMD	$\frac{MD}{\sigma MD}$	Odds
Lightning Express No. 7 and Rowden 3003.	2.177	0.10035	21.694	Infinite	0.040	0.01707	2.343	107:1.
Lightning Express No. 7 and New Boykin 1235.	2.460	.09575	25.692	do	.140	.01405	9.964	Infinite.
Rowden 3003 and New Boykin 1235.	0.283	.11719	2.415	61.7:1	.100	.01739	5.75	Do.

Table 10 presents the differences between the mean values for each of the four fiber diameter measures in each pair of the three strains. The mean values for Rowden 3003 and New Boykin 1235 are quite similar. The mean difference in width is only 0.043μ, which has no statistical significance when considered in relation to its standard deviation of the mean difference value of 0.15622. The mean difference of 0.515 in thickness is significant, the odds being 32,894:1. The mean difference of the mean diameter, $\frac{W+T}{2}$, is significant with odds of 62:1, even though the difference of $0.11719_2\mu$ is rather too small to be of probable practical value so far as predicting the spinning value of a cotton variety is concerned. The greatest statistical significance among the four dimensional measurements for Rowden and New Boykin is found in the mean difference of the ratio of fiber width to thickness, $\frac{W}{T}$, where the significance approaches infinity. This high significance might be interpreted as meaning that the calculated ratio constant is more sensitive in detecting a differential development of fiber-wall thickness between strains than either of the other constants used.

A comparison of the diameter measurements of Lightning Express No. 7 with Rowden 3003 and New Boykin 1235 brings out several interesting facts. Due to the similarity of the measurements for Rowden and New Boykin, as previously shown, it would be expected that the mean differences between Lightning Express and each of the two strains would be comparable. Such is found to be the case with the single exception of the mean ratio difference of Lightning Express

and Rowden, where the odds are only 107:1. This ratio, while statistically significant, is in poor agreement with the odds for the other comparisons, where in all cases the mean difference divided by the standard deviation of the mean difference exceeds the range of ordinary tables of odds.

SUMMARY AND CONCLUSIONS

The research of previous investigators indicates that an intimate relationship exists between fiber diameter dimensions and the spinning and manufacturing value of cotton.

Several methods of making diameter measurements have been proposed by various investigators, but among these methods for measuring fineness it seems that the measurement of mean diameter $\frac{W+T}{2}$ suggested by Barritt is the most practical from the standpoint of giving a measure of the normal collapsed fiber.

The calculation of the diameter dimension ratio $\frac{W}{T}$ is proposed for the purpose of giving a measure of the relative fiber-wall thickness among different varieties and strains. Because of the intimate relationship existing between fiber-wall thickness and convolutions and between convolutions and spinning utility, it is believed that this constant will prove to be valuable in correlating fiber characters with the quality of the manufactured product, and in predicting the value of new strains.

Five hundred measurements of width and thickness were made on each of three strains in order to determine the proper size of sample for statistical significance. The strains used were Rowden 3003, New Boykin 1235, and Lightning Express No. 7.

Frequency distributions are presented in tabular and graphic form for each of the fiber dimensional constants. These were constructed for the purpose of testing whether sufficient skewness or multimodality was present to invalidate the use of the probable error concept. A consideration of all of the distributions suggests that diameter measurements may be safely regarded as normal distributions in so far as statistical treatment is concerned.

A comparison of the frequency distributions for the diameter dimension ratio $\frac{W}{T}$ in the three strains shows a greater range in the case of Rowden 3003 than in either of the other strains tested, and the hypothesis is advanced that this may be due to a lack of physiological balance occasioned by selecting for staple length while retaining the genetic diameter of a coarse short-staple cotton.

The frequency distribution table also contains a comparison of the means and the probable error of the means as calculated by the machine method, and by a grouping method, for each of the dimensional measurements in each of the three strains. This comparison showed that significant errors were not introduced by the rounding off used in the conversion table, or by the size of class selected in constructing the frequency distributions.

Tables and graphs are presented which show the mean, the probable error of the mean, and the coefficient of variability of the mean in units of 10, from 10 to 500, for each of the four dimensional measures in each of the three strains used in this study.

A comparison of the calculated and theoretical probable errors of the mean for various size of sample is presented in tabular and graphic form. The comparison is made for each dimensional constant in each strain, in units of 50, from 50 to 500. The good agreement of the calculated with the theoretical curves indicates that the technic used in making the measurements was such that a fairly good random distribution of values was secured.

A formula is presented by the use of which the size of sample may be determined for which differences will be statistically significant within a given range of accuracy. Considering the difference in mean measurements of the varieties included in this experiment, a range of $\pm 0.5\mu$ is believed to be sufficiently limited to serve as a means of dividing strains into a number of classes of fineness. Subsequent studies of the relation of degree of fineness to manufacturing utility will enable the research worker to determine the size of sample that should be used in making an analysis of diameter measurements in order to secure any desired degree of accuracy in the results.

The theoretical size of sample which will probably reveal significant differences was calculated on the basis of a range of 2.5 per cent of the mean for each constant in the three strains.

The evidence secured from this experiment indicates that a sample size of 100 measurements of width and thickness will provide a safe margin of statistical significance for the determination of differences on the basis of a $\pm 0.5\mu$ range, as well as a range of 2.5 per cent of the mean.

Tables show the mean diameter measurements for the entire sample for each of the strains, and the mean differences between the strains. Rowden 3003 and New Boykin 1235 are similar in fineness while differing considerably in staple length. Differences of considerable magnitude were found between Lightning Express No. 7 and the other strains.

PATHOGENESIS IN THE WOOLLY-KNOT TYPE OF CROWN GALL[1]

By E. A. SIEGLER, *Pathologist*, and R. B. PIPER, *Scientific Aid, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

For many years the investigations of the crown-gall disease caused by *Bacterium tumefaciens* Smith and Town. on various hosts have been concerned mostly with phases of the problem other than control. This has probably been due to the more intriguing bacteriological and histological phases of the problem and possibly to the inherent difficulties encountered in investigations of soil-borne organisms. In problems of disease control, however, the importance of knowing the period of greatest susceptibility as well as the sources of inoculum is well recognized. This paper reports experiments which furnish evidence bearing directly on these questions in connection with the type of crown gall on apple commonly called woolly knot.

REVIEW OF LITERATURE

After Smith and Townsend (*13*)[2] isolated the crown-gall organism from the Paris daisy and other hosts, there followed a period of inactivity with respect to practical control measures. Although Smith and his associates (*12*) emphasized the differences in strains of the crown-gall organism, apparently not much significance was attached to this important question. Riker and Keitt (*7*) and Muncie (*2*) interpreted the evidence of Smith and his coworkers (*12*) as being insufficient to explain the cause of the type of malformation frequently encountered in the grafted apple nursery. They classified these malformations as "wound overgrowths," nonpathogenic in nature. Siegler (*8*) called attention to the significance of the apple strain of Smith et al. and later (*9*) offered evidence as proof that it is the cause of the woolly-knot type of crown gall. In the present paper all references, unless qualified, are made to this type of gall. Recently Riker et al. (*4*) announced the isolation of a "hairy-root" organism from malformations on apple roots. This organism, judging from its effect on various hosts, apparently is the strain of *Bacterium tumefaciens* (*1*, *10*), referred to as the apple hairy-root strain by Smith et al. (*12*), and, for convenience, as the apple strain by Siegler (*10*) and Brown (*1*).

In an attempt to explain the beneficial effect of a disinfectant applied to the stock before grafting, it was reasoned (*9*) that a considerable amount of infection might be due to the presence of the organism on the stock at the time of grafting. This idea was originally conceived by M. B. Waite, who originated the formaldehyde-solution dip for apple seedlings (*14*).

[1] Received for publication June 30, 1931; issued January, 1932.
[2] Reference is made by number (italic) to Literature Cited, p. 1002.

Riker and Keitt (*7*) and Muncie (*2*) reported that the callus on the freshly made graft was not an infection court for the crown-gall organism. However, Siegler (*9*) secured infections with the apple strain on callus of similar material, and has concluded that the failures of previous investigators to obtain infections were due to the difference in the strains of the organisms used, since these workers evidently were not using the apple strain. It was also strongly indicated that although the callused graft was only moderately susceptible to infection with the apple strain the graft union before callusing was extremely susceptible. This fact led to emphasizing the importance of keeping down infection during the grafting process. It served to explain the partial control of malformations secured by Waite and Siegler (*14*) when the seedlings were dipped, before grafting, in a formaldehyde solution or in an organic mercury solution and indicated that a favorable line of attack in controlling infections lies in protecting the host during the grafting period.

Riker et al. (*6*) suggest that the critical period for infection by their hairy-root organism does not occur at the time the grafts are made, but after the middle of June. Since their work has been published only in abstract form, it is not known what attempts, if any, were made to obtain infection at the time of grafting.

The data previously presented by the senior writer (*9*) on the critical period for infections are supplemented in this paper by similar experiments designed to subject the evidence already obtained (*9*) on this point to a more critical examination. Other experiments, intended especially to furnish direct evidence as to the source of infectious material in nature, are also reported.

EXPERIMENTS

EFFECT OF CALLUS FORMATION ON THE SUSCEPTIBILITY OF THE GRAFT UNION TO THE APPLE STRAIN OF THE CROWN-GALL ORGANISM

In the following experiments the grafts in each series were made as uniformly as practicable, but the unions were not perfectly matched. In the tables, under the heading "Host," the scion variety is given first and is followed by the source of the seed from which the seedlings were grown. The seedlings were grown in the United States. In the experiments, the results of which are given in Tables 1 to 3, the seedling understocks were disinfected with a 1 to 400 solution of an organic mercury compound, which was washed off with tap water before the seedlings were cut up for grafting, and the grafts were wrapped with raffia. In all the experiments the trees were examined after they had made one season's growth (1929). Unless otherwise specified, the apple strain of the crown-gall organism was used as the inoculum.

In one experiment the grafts were inoculated at the time they were made, either by smearing the culture from an agar slant on the lower end of the cut surface of the scion or by immersing the grafts for a short time in a water suspension of the culture. (Table 1.) Approximately 350 trees grown from grafts inoculated with the apple strain and 275 trees grown from noninoculated grafts were examined. Ninety-four per cent of those grown from the inoculated grafts were infected; 15 per cent of those grown from the noninoculated grafts were infected. The malformations in all cases were typical woolly knots, as shown in Figure 1, A, which illustrates trees

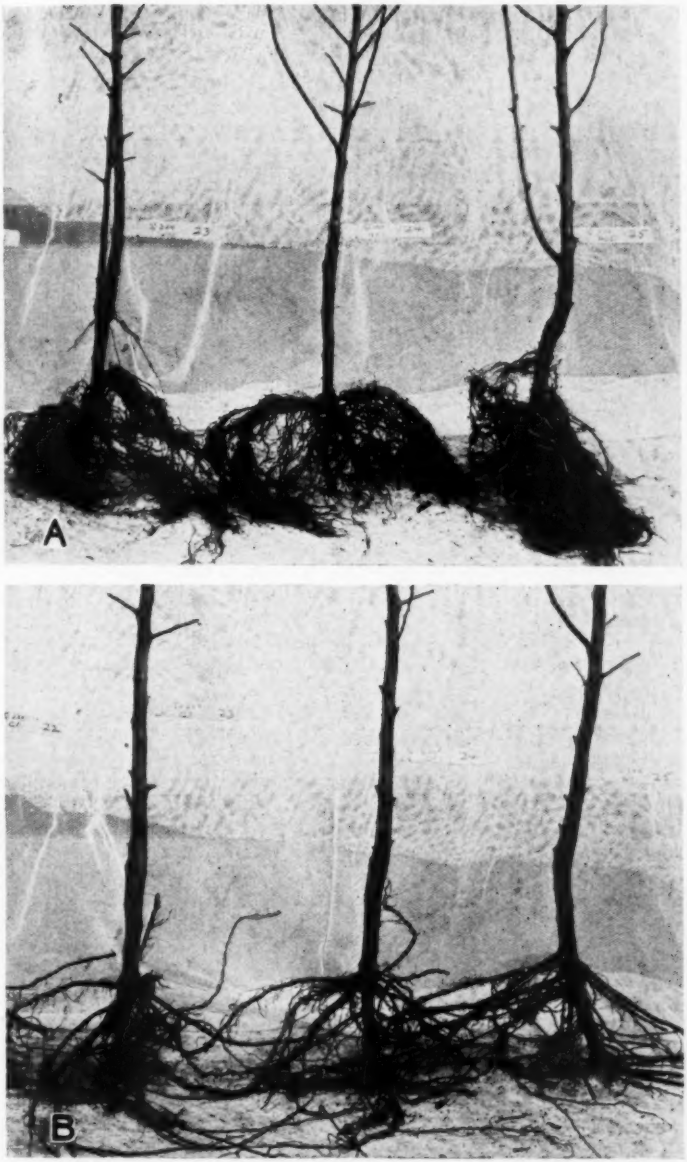

FIGURE 1.—Typical trees grown from the grafts in experiment 224: A, Woolly knots resulting on all trees grown from grafts inoculated with the apple strain; B, smooth unions resulting on 35 per cent of the trees grown from noninoculated grafts. Greatly reduced

of an unknown variety grown from inoculated grafts. Trees grown from the noninoculated grafts, typical of those classed as clean, are shown in Figure 1, B. The entire lots of trees of the same unknown variety, obtained from the inoculated and noninoculated grafts in experiment 224, are shown in Figure 2, A, and Figure 2, B, respectively. The trees grown from this inoculated lot of grafts are distinctly reduced in size as compared with those grown from the noninoculated controls. A reduction in size was apparent in all the inoculated lots.

Although in this paper the effect of the apple strain is considered almost exclusively, the results of inoculations with other strains of the crown-gall organism are also shown in Table 1. The so-called smooth gall strains, such as the peach, daisy, and pear, produce the smooth type of gall on the apple (*9, fig. 2*) and especially on the stone fruits. However, in some cases a considerable percentage of malformations typical of woolly knots resulted in trees grown from grafts inoculated with these strains. This has been interpreted (*9*) as probably caused by natural infections with the apple strain, although there is a probability of mixed cultures, especially in one case. However, the essential fact to be noted is that there is no apparent reason to question the purity of the cultures of the apple organism which has been used throughout all the writers' experiments on woolly knot. Cultures of this organism have invariably produced the woolly-knot type of gall, and it is upon the results obtained with this organism that these investigations, as well as past reports, have been based. The cultures of other strains have been used merely to illustrate the type of malformations produced by strains other than the apple (hairy root) strain.

In another series of experiments certain lots of grafts were inoculated when made, by immersing them in a suspension of a culture of the apple strain in sterile water. Other lots were allowed to callus for 54 days and were then inoculated in the same manner. The results of the inoculations are given in Table 2. Of the grafts inoculated when made, 81 per cent became infected; of those inoculated after callus had formed, 66 per cent became infected. The noninoculated control grafts showed 33 per cent and 18 per cent infected, respectively. These results are in agreement with those previously recorded, but are not so conclusive (*9*), because a relatively high percentage of infection occurred on the controls and on the grafts inoculated after they had callused. In similar experiments, to be discussed later, infection on the controls was practically eliminated.

FIGURE 2.—Entire lots of trees grown from the grafts in experiment 224: A, Trees grown from grafts inoculated with the apple strain; B, trees grown from noninoculated grafts. Greatly reduced

TABLE 1.—*Susceptibility of the graft union as shown by inoculations with the apple and other strains, made before callusing*

Experiment No.	Host	Inoculum	Grafts planted	Grafts surviving	Stand	Trees showing galls of indicated type					
						Smooth		Woolly knot		Total	
			Number	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
210-A (control)	Paragon on Vermont	Not inoculated	125	77	61.6	0	0	0	0	0	0
210-B	do	Daisy (peach) strain (BT-102) smeared on scion tip.	125	85	68.0	7	8.2	1	1.2	8	9.4
210-C	do	Daisy (peach) strain (491-108) smeared on scion tip.	125	80	64.0	37	46.3	0	0	37	46.3
210-D	do	Apple strain (486-107) smeared on scion tip.	125	77	61.6	0	0	74	96.1	74	96.1
210-E	do	Pear strain (637-109) smeared on scion tip.	125	74	59.2	17	23.0	5	6.8	22	29.7
211-A (control)	Tolman Sweet on French	Sterile water	125	64	51.2	0	0	26	40.6	26	40.6
211-B	do	Daisy (peach) strain (BT-102) water suspension.	125	91	72.8	3	3.3	25	27.5	28	30.8
211-C	do	Apple strain (486-107)	125	89	71.2	0	0	84	94.4	84	94.4
211-D	do	do	125	67	53.6	0	0	59	88.2	59	88.1
211-E	do	Daisy (peach) strain (491-108)	125	76	60.8	63	82.9	0	0	63	82.9
211-F	do	Pear strain (637-109)	125	79	63.2	11	13.9	10	12.7	21	26.6
211-G	do	do	125	72	57.6	28	38.9	28	38.9	56	77.8
239-A (control)	Williams on French	Sterile water	84	61	72.6	0	0	1	1.6	1	1.6
239-B	do	Apple strain (486-107)	84	62	73.8	0	0	56	90.3	56	90.3
239-C	do	Daisy (peach) strain (491-108)	84	67	79.8	18	26.9	0	0	18	26.9
239-D	do	Pear strain (637-109)	84	80	95.2	26	32.5	0	0	26	32.5
224 (control)	Unknown on Northern Spy 227 (clone).	Not inoculated	50	45	90	0	0	6	13.3	6	13.3
224	do	Apple strain (486-107) smeared on scion tips.	50	40	80	0	0	40	100	40	100
246 (control)	Cortland on Northern Spy	Not inoculated	34	27	79.4	0	0	8	29.6	8	29.6
246	do	Apple strain (486-107)	34	31	91.2	0	0	31	100	31	100
Total or average		Controls	418	274	65.6	0	0	41	15.0	41	15.0
		Smooth-gall strains	1,043	704	67.5	210	29.8	69	9.8	279	39.6
		Apple strain (486-107)	543	366	67.4	0	0	344	94.0	344	94.0

TABLE 2.—*Susceptibility of the graft union as shown by inoculations with the apple strain, made before and after callusing*

[Early Ripe variety on seedlings grown from Vermont seed]

Experiment No.	Inoculum	Inoculum applied before or after callusing	Grafts planted	Grafts surviving	Stand	Trees showing woolly-knot galls at graft union	
			Number	*Number*	*Per cent*	*Number*	*Per cent*
209-A	Control (sterile water)	Before	125	103	82.4	38	36.9
209-B	Apple strain (486-107)	do	125	87	69.6	79	90.8
209-E	Control (sterile water)	After	125	117	93.6	17	14.5
209-F	Apple strain (486-107)	do	125	110	88	63	57.3
209-C	Control (sterile water)	Before	125	64	51.2	18	28.1
209-D	Apple strain (486-107)	do	125	88	70.4	64	72.7
209-G	Control (sterile water)	After	125	93	74.4	21	22.6
209-H	Apple strain (486-107)	do	125	95	76	73	76.8
Total or average	Controls (sterile water)	Before	250	167	66.8	56	33.5
	Apple strain (486-107)	do	250	175	70	143	81.7
	Controls (sterile water)	After	250	210	84	38	18.1
	Apple strain (486-107)	do	250	205	82	136	66.3

Perhaps the most critical experiment relating to the question of susceptibility of the graft union was performed when the graft unions of the Jonathan variety were inoculated at different stages of callus formation. Ninety-four per cent of the grafts became infected when they were inoculated at the time of grafting. (Table 3.) The degree of susceptibility decreased with the progress of callus formation so that only 11 per cent of those grafts that had been allowed to callus for 43 days before they were inoculated became infected. Of the corresponding noninoculated control grafts, 8 and 1 per cent, respectively, became infected. Although emphasis should not be placed on this single experiment of one year's duration, the results strongly suggest that the development of callus progressively lessens the liability to infection.

TABLE 3.—*Influence of callus formation on susceptibility as shown by inoculations with the apple strain, made on Jonathan apple grafts*

Time of inoculation	Method of inoculation	Grafts planted	Trees dug	Stand	Trees clean	Trees showing woolly-knot galls			
						Total	At root tip	At union	
		Number	*Number*	*Per cent*	*Number*	*Number*	*Number*	*Number*	*Per cent*
At time of grafting	Immersed in sterile water 1 hour (control).	125	74	59.2	68	6	0	6	8.1
Do	Water suspension apple strain for 1 hour.	125	86	68.8	1	85	6	81	94.2
24 days after grafting	do	125	125	100	4	121	23	109	87.2
35 days after grafting	do	125	112	89.6	46	66	25	44	39.3
43 days after grafting	do	125	115	92	75	30	19	13	11.3
Do	Sterile water 1 hour (control).	125	101	80.8	100	1	0	1	1

Thus further evidence that the graft union is extremely susceptible to infection at the time the grafts are made is added to that previously reported (*9*). In addition, the correlation of increasing resistance with the progressive formation of callus offers a reasonable explanation as to why the graft union is most susceptible previous to callusing.

EFFECT OF REMOVING SURFACE-BORNE ORGANISMS FROM THE SEEDLING UNDERSTOCK

An experiment was made similar to one previously reported (*9*) wherein a disinfectant was applied at each of the following stages: (1) Scions and stocks before cutting for grafting, (2) newly made graft after wrapping, and (3) graft after callusing, just before planting. In confirmation of previous results (*9*), it is evident from the data in Table 4 that the most effective application of a disinfectant is that made on the scions and stocks before grafting. By this application the percentage of infection was reduced from 30 to 2. As would be anticipated, a disinfectant applied after the grafts had callused was not so effective in preventing the malformations. Obviously, if the grafts before callusing are extremely susceptible to infection and the inoculum is present, a disinfectant applied after callus has formed will not be efficacious. These results, strictly in accord with those of previous experiments, also furnish evidence that the seedling understocks carry the inoculum as surface-borne organisms. Isolations from these understocks have yielded the organism in culture.

TABLE 4.—*Results of disinfecting Tolman Sweet apple grafts at different periods*

Treatment	Grafts planted	Trees dug	Stand	Trees clean	Trees showing galls	
	Number	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
Not washed (control)	100	97	97.0	68	29	29.9
Scions and stocks disinfected before grafting	132	127	96.2	124	3	2.4
Grafts disinfected before callusing	120	113	94.2	102	11	9.7
Grafts disinfected after callusing	127	123	96.9	96	23	18.7

The assumption that the seedlings might carry superficially surface-borne organisms in significant numbers was subjected to the following test: Grafts were grown (1) from seedlings that had been very thoroughly washed, (2) from seedlings that were thoroughly washed and also disinfected, and (3) from seedlings that were not washed or disinfected but merely cut up for grafting and handled according to commercial practice.

The details of this experiment are given in Table 5, where it is seen that the grafts grown from the seedlings that were neither washed nor disinfected showed 48 per cent infection as compared with 8 per cent infection on grafts grown from seedlings that were very thoroughly washed, presumably removing surface-borne organisms, but not disinfected. When the seedlings were disinfected after washing, the amount of infection was reduced to less than 1 per cent.

The results obtained by a slight modification of this experiment (Table 5, lots 12, 13, and 14) were strictly in accord with those of previous experiments in that 41 per cent infection resulted on grafts grown from seedlings that were given only a superficial washing but no treatment, compared with less than 1 per cent infection on grafts grown from seedlings that were washed and also disinfected. Thus tangible evidence is furnished that the seedling understocks are a most important source of infection.

TABLE 5.—*Comparison of the susceptibility of grafts made from washed and disinfected apple seedlings with that of grafts made from seedlings which were neither washed nor disinfected*

Lot No.	Treatment of—		Grafts planted	Grafts surviving	Stand	Trees showing woolly-knot galls	
	Scions and stocks	Grafts when made					
			Number	*Number*	*Per cent*	*Number*	*Per cent*
9	Not washed, not disinfected	Not treated	100	92	92.0	44	47.8
10	Washed thoroughly, not disinfected.	do	125	122	97.6	10	8.2
11	Washed and disinfected	do	110	105	95.5	1	1.0
12	Superficially washed, not disinfected.	do	116	109	94.0	45	41.3
13	Washed and disinfected	do	125	112	89.6	1	.9
14	do	Disinfected	125	108	86.4	4	3.7

EFFECT OF EXPOSING THE SEEDLING UNDERSTOCKS BY IMMERSING THEM IN A WATER SUSPENSION OF THE INOCULUM

This series of experiments was designed to simulate conditions that would presumably obtain if the stock were grown in heavily infested soil. The supposition that the seedlings may often be contaminated with this organism, even in the absence of any sign of infection, was the obvious one as the result of the experiments previously noted (*9*, *14*). All the seedlings were given a superficial washing. Two lots were used as noninoculated controls and two lots were exposed to the organism by immersing them in a sterile water suspension of the apple strain. Each lot of noninoculated control seedlings was divided into two lots, in one of which the seedlings and the grafts were disinfected at the time of grafting. Each lot of inoculated (exposed) seedlings also was divided into two lots, in one of which the seedlings and the grafts were disinfected at the time of grafting. All the seedlings were stored away for one month before they were used for grafting.

The results given in Table 6 show that 59 per cent of the grafts made from the seedlings that were exposed to the inoculum without subsequent disinfection became infected, and that 10 per cent of the grafts whose seedlings were exposed to the inoculum but were disinfected one month later, at the time of grafting, became infected. The noninoculated control seedlings that were not disinfected produced grafts showing 36 per cent infection, whereas when the other lot of noninoculated control seedlings was disinfected before grafting and the newly made grafts were also disinfected, only 1 per cent of the resulting grafts showed infection.

The evidence from this experiment indicates that the increase in the amount of infection on the grafts grown from seedlings exposed to the inoculum was due to the organisms placed on the seedlings. Organisms can apparently remain virulent on the uninjured surfaces of seedlings in storage for at least one month previous to grafting.

TABLE 6.—*Effect of exposure (by immersion) of seedlings to the apple strain before grafting*

Experiment and lot No.	Host	Inoculations on stock	Treatment of—		Grafts planted	Trees dug	Stand	Trees showing gall at graft union	
			Stocks	Grafts when made					
					Number	*Number*	*Per cent*	*Number*	*Per cent*
Experiment 201, lot 1	Williams on Jonathan seedlings.	Not inoculated	Untreated	Untreated	41	29	70.7	16	55.2
Experiment 201, lot 2	do	do	Semesan	Semesan	45	38	84.4	1	2.6
Experiment 201, lot 3	do	Inoculated with apple strain (486–107)	Untreated	Untreated	54	45	83.3	40	88.9
Experiment 201, lot 4	do	do	Semesan	Semesan	53	44	83.0	0	0
Experiment 241, lot A–1	Yellow Transparent on Vermont seedlings.	Not inoculated	Untreated	Untreated	108	100	92.6	31	31.0
Experiment 241, lot A–2	do	do	Semesan	Semesan	121	102	84.3	1	1.0
Experiment 241, lot B–1	do	Inoculated with apple strain (486–107).	Untreated	Untreated	121	111	91.7	52	46.8
Experiment 241, lot B–2	do	do	Semesan	Semesan	121	102	84.3	15	14.7
Total or average		Not inoculated	Untreated	Untreated	149	129	86.6	47	36.4
		do	Semesan	Semesan	166	140	84.3	2	1.4
		Inoculated with apple strain (486–107).	Untreated	Untreated	175	156	89.1	92	59.0
		do	Semesan	Semesan	174	146	83.9	15	10.3

SEASONAL DEVELOPMENT OF GALLS

An experiment which does not bear directly on the main points in this paper was made to study the effect of inoculations at intervals throughout the growing season. The inoculations were made by inserting the organism into horizontal slits on the scion approximately one-quarter of an inch above the graft union. The detailed results are given in Table 7, where it is seen that practically 100 per cent infection was secured on the inoculated trees and that none of the noninoculated control slits became infected despite the wound. Although the number of trees used was small, it should be noted that natural infection, under the conditions of this experiment, did not occur during the growing season from May 23 to August 29, even when a wound was made to permit infection from the soil. In accord with results previously obtained (*9*), the galls produced from inoculations made on August 29 were almost as large as those obtained from inoculations made on May 23.

TABLE 7.—*Inoculations on the Yellow Transparent variety*

Inoculum	Date of inoculation	Trees inoculated	Trees surviving	Trees showing galls	
		Number	*Number*	*Number*	*Per cent*
Apple strain (486–107)	May 23, 1929	10	10	10	100
Not inoculated	do	10	10	0	0
Apple strain (486–107)	June 6, 1929	15	14	14	100
Not inoculated	do	15	13	0	0
Apple strain (486–107)	June 28, 1929	15	15	14	93.3
Not inoculated	do	15	15	0	0
Apple strain (486–107)	July 12, 1929	15	15	15	100
Not inoculated	do	15	15	0	0
Smooth-gall strain (671)	do	15	15	14	93.3
Apple strain (486–107)	Aug. 3, 1929	10	8	8	100
Not inoculated	do	10	10	0	0
Apple strain (486–107)	Aug. 15, 1929	10	10	10	100
Not inoculated	do	10	10	0	0
Apple strain (486–107)	Aug. 29, 1929	18	17	15	88.2
Not inoculated	do	18	18	0	0

Incidentally, it is again noted that inoculations with a strain apparently similar to the daisy or peach strain of Smith et al. (*12*) resulted in the formation of smooth galls only. Figure 3, A, represents a typical smooth gall resulting from an inoculation with an organism obtained from a similar-appearing smooth gall on apple. This, of course, is the type of gall which Riker and Keitt (*7*) and Muncie (*2*) designate as "true crown gall." All the galls produced in this experiment by inoculations with the apple strain, however, were typical woolly knots.

DISCUSSION

Since this paper was written two additional reports (*5*, *15*) have appeared. Riker et al. (*5*) report the isolation of the hairy-root organism from malformations, including "woolly knots," and have named this organism *Phytomonas rhizogenes*. These workers induced "hairy root" by inoculations with this organism on the underground parts of apple. There is apparently no reason to doubt that this organism is the apple or hairy-root strain of *Bacterium tumefaciens* used in the experiments described herein. Wright et al. (*15*) stress the importance of using single-cell isolations to secure pure cultures. In the writers' experiments the purity of the cultures used in all

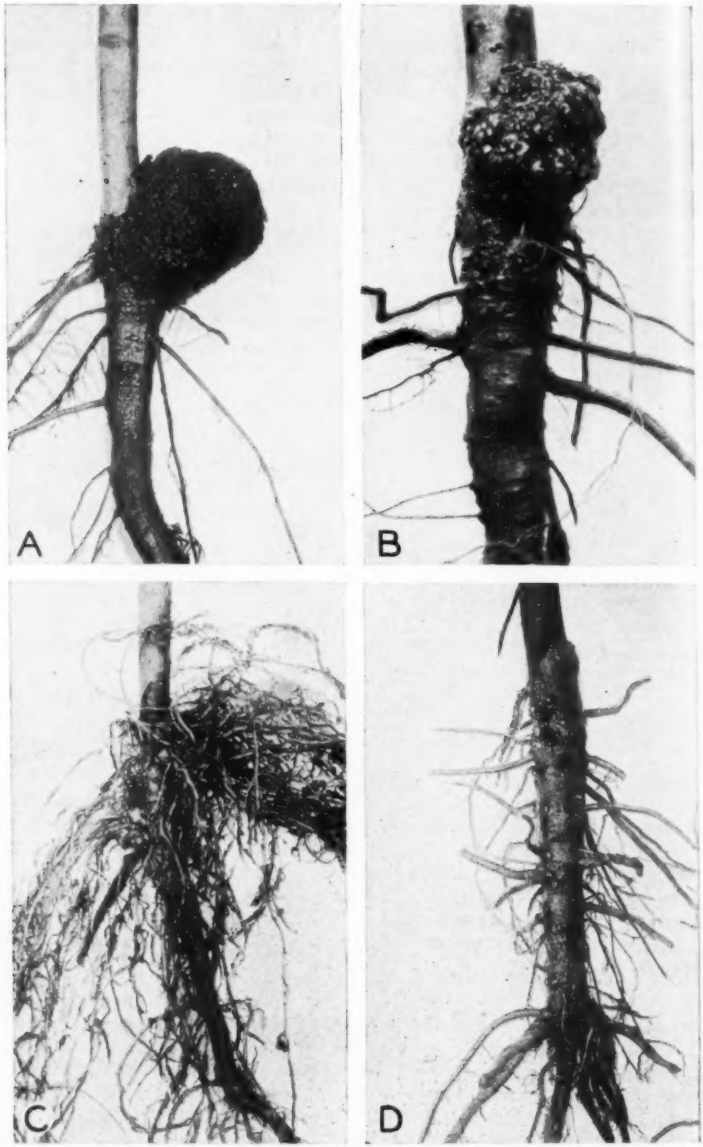

FIGURE 3.—A, Smooth type of gall on Paragon caused by a strain probably identical with the daisy (peach) organism, obtained from a similar-appearing apple gall. × ½. B, Type of malformation found on the control lots of Cortland grafts in experiment 246, natural size. C, Typical woolly-knot type of gall obtained on 96 per cent of the trees grown from grafts of the Paragon variety in experiment 210, inoculated with the apple strain. × ½. D, Typical smooth unions obtained on all the trees grown from the noninoculated grafts of the Paragon variety in experiment 210. × ½.

critical experiments with the apple strain has been attested by the fact that thousands of inoculations on the aboveground parts of various hosts have invariably resulted in differential tests that were specific for the organism used. Thus the final criterion for these tests was the same as that employed by Wright et al. (*15*) to test the purity of their single-cell isolations.

Much of the data presented herein has been obtained as the result of experiments of only one year's duration, but some of the experiments are either modifications or repetitions of previous experiments bearing directly on the question of the susceptibility of the newly made graft union to the apple strain of the crown-gall organism. The number of grafts used and the stands obtained, together with the consistent results, are sufficient to furnish reliable evidence on the important questions of the source of the inoculum in nature and the critical period of host susceptibility.

Before entering into a discussion of these two phases of the problem an attempt will be made to state the general problem. This will necessitate a somewhat detailed discussion of the recent contributions of Riker and his coworkers (*6*), Muncie and Suit (*3*), and Brown (*1*). The last named has corroborated the findings of Smith et al. (*12*) as to the effect of inoculations at the collars of apple trees. Muncie and Suit (*3, p. 296*) summarize their results in part by stating: "True crown-gall * * * occurred on an average of less than 1 per cent * * *." These writers also report the isolation of 10 organisms "which closely resembled young colonies of *Ps.*[*eudomonas*] *tumefaciens* in plate culture" from "young apple seedlings showing the burr-knot type of hairy root and from young grafted apple trees with fleshy roots arising from overgrowths upon the scion." Inoculations into young apple shoots below ground produced malformations characterized by "the development of an abundance of fleshy roots from overgrowths on the apple." A comparison of the second and third specimens in Plate 5, A, of Muncie and Suit (*3*) with Figure 1 of Siegler (*8*) shows the similarity, as these workers suggest, between the malformations produced by their hairy-root organism and those produced by the apple strain of the crown-gall organism. Muncie designates this organism the "hairy-root organism." Although inoculations apparently were not made on the graft union, these writers state: "The formation of overgrowths at the union of piece-root grafted trees may be accounted for largely on the basis of wounding, as the result of grafting, and the imperfect fitting of scion and stock * * *."

Riker et al. (*4*) also have recently reported the isolation of an organism resembling *Bacterium tumefaciens* from "naturally occurring malformations" on apple roots, and refer to it as the "hairy-root organism." As stated, this organism apparently is identical with the apple strain (*1, 8*) and will be considered as such in this discussion. These workers report inoculations with this organism on nursery apple trees. From their text it is presumed that inoculations were not made on the graft union at the time of grafting. The importance of making such inoculations can hardly be overemphasized.

Riker and Keitt (*7, pl. 39, figs. A–D*) had previously illustrated what they considered typical wound overgrowths. These malformations were similar to, if not identical with, those from which Siegler (*8, fig. 1*) isolated the apple organism and, by means of suitable

controls, proved its pathogenicity on the apple. The constant association of an organism with a host on which it may become pathogenic does not, however, unqualifiedly prove it to be the cause of any abnormal condition. Perhaps the critical test is the absence of these malformations in the apparent absence of the organism, assuming all other conditions to be uniform. In dealing with the question of callus at the graft union there are stages in which it is difficult to draw a hard-and-fast line between normal and abnormal callus formation preceding the healing of a wound. Naturally, there are various gradations in normal wound healing, and those cases in which the callus or wound tissue does nor form a comparatively smooth union after a somewhat indefinite period, approximately one year, may be spoken of as wound overgrowths. When the fact is noted that wound tissue may be formed under varied conditions of environment and of the genetic composition of the two elements of the host, and when there are added to these the further complications incident to infection, such as the amount of inoculum involved and the condition of the host before and after infection, it is evident that innumerable variations will occur, especially in the pathogenic malformations. It is conceivable that many malformations may be initiated as excessive callus formations which are later invaded by the organism; and it is equally possible that many may be initiated by the abnormal growth of the organism, which may later die or at least can not be readily recovered, as in the case of certain aerial galls (*11*). In experiments previously reported (*9*) and in the experiments shown in Tables 1, 2, and 3, it is desired, therefore, to emphasize the significance of the absence in some cases, and the pronounced reduction in all cases, of malformations at the graft union when precautions were taken to exclude the organism. The failure to obtain overgrowths in the apparent absence of the organism is considered evidence of a more convincing nature than the ability to produce overgrowths at will by inoculations.

It is not unusual for 90 per cent of the trees resulting from grafts inoculated on the union at the time of grafting to show malformations similar to those in Figure 1, A, and for trees resulting from non-inoculated grafts to be practically free from malformations. At least 50 per cent more galls have always been obtained on the inoculated than on the noninoculated lots. Comparable results were obtained with the Cortland, which is by far the most susceptible apple variety known to the writers. In the experiment of which the results are shown in Table 1, the scions and stocks were disinfected and the disinfectant washed off before grafting. Inoculations on one lot of these grafts (experiment 246) were made by smearing the culture on the lower tip of the scion; one lot was used as controls. The graft unions of the different lots were made as uniformly as possible, but were not perfectly matched. All the trees in the inoculated lot bore galls, whereas only 30 per cent of the trees in the noninoculated lot were galled. These malformations were similar to the specimen illustrated in Figure 3, B. No isolations were attempted from the malformations on trees in the control lots, but isolations from the malformation illustrated yielded the apple strain.

This type of woolly knot, practically free from roots, was not frequently produced by inoculations in these experiments. However, the assumption that it was the result of natural infection seems warranted, especially when the variations in the amount of the inocu-

lum, in varietal reaction, and in the condition of the host during the incubation stage following natural inoculation are considered in comparison with artificial inoculations made under more uniform conditions.

The results obtained on the Paragon variety (Table 1) present more convincing evidence. Here 96 per cent of the 77 trees grown from the inoculated grafts showed malformations, as illustrated in Figure 3, C, whereas all the trees grown from the noninoculated grafts, of which a typical one is illustrated in Figure 3, D, were free from malformations. Wound overgrowths caused by poorly fitting unions, by uncongenial scions and stocks, by environmental conditions, or by any other cause, as, for example, girdling by a wire above the union (fig. 4), should appear with approximately the same frequency on trees grown from uniform lots of grafts on uniform soil. When malformations do not appear on noninoculated grafts, as in this case, there is every reason to question why they should occur in significant numbers in commercial plantings. Even when the factor of nonuniformity of the understock was eliminated, as in experiment 224, only 13 per cent of the trees in the control lot bore malformations. Although in one control lot (experiment 211) 40 per cent of the trees showed malformations, this was an exceptional case and, as compared with the 95 per cent of infections obtained on the trees grown from the inoculated grafts, this percentage was relatively low. In other experiments (Tables 4 and 5) in which the grafts were not only made aseptically but also contained a coating of the disinfectant on their surfaces when they were stored for callusing, the number of malformations on the resulting trees was negligible.

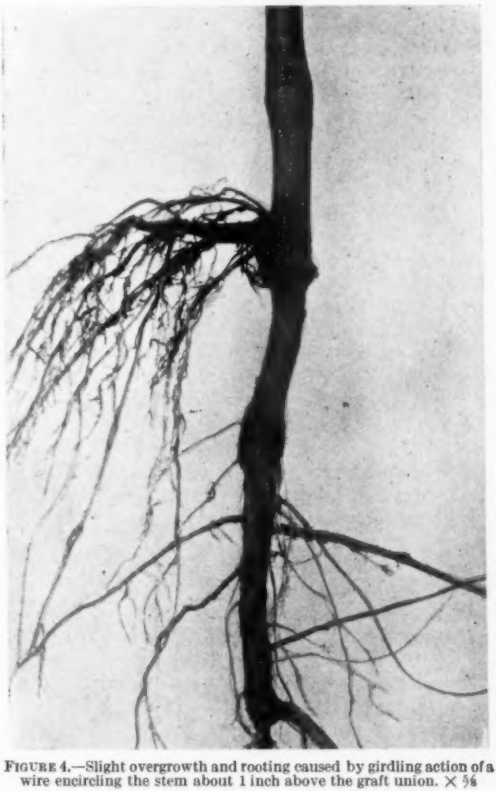

FIGURE 4.—Slight overgrowth and rooting caused by girdling action of a wire encircling the stem about 1 inch above the graft union. × ⅝

A summation of the data in Tables 1, 2, and 3 shows that 90 per cent of the 627 trees resulting from grafts that were inoculated with the apple strain when made bore malformations and that only 20 per cent of the 515 trees growing from the noninoculated control grafts bore malformations. Although the grafts in these experiments were made as aseptically as possible, they obviously were subject to infection from the time they were stored for callusing. The conclusion that practically all the malformations on the controls were the result of natural infection seems justified, especially in view of the results of similar experiments in 1928 (*9*) in which no malformations appeared on the controls.

The fact that no disinfectant was used on the surface of these grafts (Tables 1, 2, and 3) or on the wrappers precludes the suggestion properly made by Riker and Keitt (*7*) that malformations in certain previous experiments were inhibited or retarded either because of injury to the tissues, which would prevent normal callusing, or by purely mechanical means. In this connection it may be said that the reason why the tape wrap for the grafts, as recommended by Riker and Keitt (*7*), gives control requires further study.

Preliminary experiments indicate that the tape wrap does not afford control when used on grafts made from seedlings that have been previously exposed by immersing them in a water suspension of the organism. However, the surfaces of these seedlings probably held larger quantities of the organism than would be expected under normal conditions. A possible explanation for the control by the tape wrap when only comparatively small numbers of the organisms are present on the seedlings is that it affords a mechanical binding which effects a speedy callus or "knitting" of the union.

Under the conditions of these experiments it is strongly indicated, if not conclusively proved, that the seedling stocks may be an important source of infection. The amount of visible infection on seedlings grown in regions where the apple strain of the crown-gall organism is prevalent is surprisingly small. That the organisms, however, are present on the surfaces in significant quantities and can be removed by a disinfectant, or even mechanically by means of a very thorough washing with water, is a conclusion justified by the evidence of experiments, the results of which are shown in Tables 4, 5, and 6.

The variation in the amount of natural inoculum on the surface of seedlings should be given consideration when interpreting results of experiments on control. It is again emphasized that the pertinent data, reported here and previously (*9*), were obtained from experiments in which the seedlings used for grafting were treated so as to give them a degree of uniformity with respect to the presence or absence of the inoculum. This procedure permits the drawing of more definite conclusions, and the results seem to offer an explanation for variations in control in field experiments.

Additional evidence is presented that the graft union is an infection court for an indefinite period during callusing after the grafts are made. The union is evidently most susceptible at the time of grafting—that is, previous to callus formation. Riker et al. (*6*) have recently suggested that the critical period for infection with the hairy-root organism does not occur at the time the grafts are made but after the middle of June. These writers also report that infections were found to occur only through wounds which ordinarily

remained as open infection courts for only two days. If, as these workers suggest, infection does not take place at the time of grafting and little if any infection is secured through uninjured callus, it is hardly probable that wounds at the graft union would be present after the middle of June in numbers sufficient to account for a high percentage of infection. It appears more plausible to account for a heavy infection by assuming a high initial infection at the time of grafting. It is significant that, in the experiments shown in Table 7, when wounds were made on 93 trees during the growing season, no infections resulted; however, since the callused grafts are slightly susceptible to infection, there are undoubtedly certain conditions that would favor abundant infections for an indefinite period from the time the grafts are planted.

It is believed that a collation of facts and hypotheses, from the standpoint of both the host and of the organism, shows that the newly made graft is an ideal locus of infection. The tissues are comparatively rich in stored food materials; the freshly cut surfaces are exposed to the organism; the grafts are stored under conditions moist enough to keep them from drying out, and at low temperatures which permit vigorous growth of the apple strain.

It is of interest to record observations made in Washington and Oregon during the tree-digging season of 1929. Malformations were extremely rare, with the exception of those definitely caused by the strain or strains of the crown-gall organism producing the smooth type of gall. Presumably, the infrequent occurrence of woolly knots in this region is due to the scarcity of the apple strain in nature; at least it is more plausible to assume this than to ascribe the scarcity of woolly knots to superior grafting technic coupled perhaps with environmental conditions which would preclude "wound overgrowths."

SUMMARY AND CONCLUSIONS

Experiments on apples are reported in which a relatively high percentage of infection occurs as a result of inoculating the graft union with the apple strain and with other strains of the crown-gall organism, *Bacterium tumefaciens* Smith and Town. (*13*). Results obtained by inoculating the graft union with the apple strain at the time of grafting and at intervals during the callusing period, indicate that the grafts are most susceptible at the time they are made and become increasingly resistant with the progression of callus formation.

Inoculations with the apple strain resulted in malformations which were invariably of the woolly-knot type. Inoculations with pure cultures of strains isolated from the smooth type of crown gall, more frequently found on the stone fruits, generally resulted in malformations of the same smooth type, but natural contaminations with other strains occurred.

When the seedling understocks were immersed in a water suspension of the apple organism one month before grafting, the trees showed a relatively high percentage of galls. When seedling understocks were very carefully washed with water before grafting, the trees were practically free from malformations; whereas, when the understocks were not washed the trees showed a high percentage of infection. These results, fortified by isolations, indicate that the seedlings may in nature carry surface-borne organisms in quantities sufficient

to be considered an important source of inoculum, especially since the graft union is exposed at the period of its greatest susceptibility and under conditions apparently ideal for incubation of the apple organism.

The possibility that seedlings may act as carriers of the inoculum should be taken into consideration when interpreting results of experiments on control either by disinfectants or by wraps.

LITERATURE CITED

(1) Brown, N. A.
1929. The tendency of the crown-gall organism to produce roots in conjunction with tumors. Jour. Agr. Research 39: 747–766, illus.

(2) Muncie, J. H.
1926. A study of crown gall caused by pseudomonas tumefaciens on rosaceous hosts. Iowa State Col. Jour. Sci. 1: [67]–117, illus.

(3) ——— and Suit, R. F.
1930. Studies of crowngall, overgrowths and hairyroot on apple nursery stock. Iowa State Col. Jour. Sci. 4: 263–313, illus.

(4) Riker, A. J., Banfield, W. M., Wright, W. H., and Keitt, G. W.
1928. The relation of certain bacteria to the development of roots. Science (n. s.) 68: 357–359.

(5) ——— Banfield, W. M., Wright, W. H., Keitt, G. W., and Sagen, H. E.
1930. Studies on infectious hairy root of nursery apple trees. Jour. Agr. Research 41: 507–540, illus.

(6) ——— Hildebrand, E., and Keitt, G. W.
1930. Seasonal development of crown gall and hairy root. (Abstract) Phytopathology 20: 124.

(7) ——— and Keitt, G. W.
1926. Studies of crowngall and wound overgrowth on apple nursery stock. Phytopathology 16: 765–808, illus.

(8) Siegler, E. A.
1928. Studies on the etiology of apple crown gall. Jour. Agr. Research 37: 301–313, illus.

(9) ———
1929. The woolly-knot type of crown gall. Jour. Agr. Research 39: 427–450, illus

(10) ———
1930. Effect of the apple strain of the crown-gall organism on root production. Jour. Agr. Research 40: 747–753, illus.

(11) ——— and Piper, R. B.
1929. Aerial crown gall of the apple. Jour. Agr. Research 39: 249–262, illus.

(12) Smith, E. F., Brown, N. A., and Townsend, C. O.
1911. Crown-gall of plants: its cause and remedy. U. S. Dept. Agr., Bur. Plant Indus. Bul. 213, 215 p., illus.

(13) ——— and Townsend, C. O.
1907. A plant tumor of bacterial origin. Science (n. s.) 25: 671–673.

(14) Waite, M. B., and Siegler, E. A.
1926. A method for the control of crown gall in the apple nursery. U. S. Dept. Agr. Circ. 376, 8 p., illus.

(15) Wright, W. H., Hendrickson, A. A., and Riker, A. J.
1930. Studies on the progeny of single-cell isolations from the hairy-root and crown-gall organisms. Jour. Agr. Research 41: 541–547, illus.

THE FASTING METABOLISM OF CATTLE AS A BASE VALUE OF HEAT PRODUCTION IN THE DETERMINATION OF THE NET ENERGY OF FEEDING STUFFS [1]

By E. B. FORBES, *Director*, WINFRED W. BRAMAN, MAX KRISS, and R. W. SWIFT, *Associates in Animal Nutrition*, with the collaboration of R. C. MILLER, R. B. FRENCH, T. V. LETONOFF, and G. R. SHARPLESS, *Institute of Animal Nutrition, Pennsylvania State College*

INTRODUCTION

The net or useful energy of foods for cattle is the gross energy, or heat of combustion of the food, minus the energy of the excreta (urine, feces, and methane) and the energy which is expended, directly and indirectly, as a result of food utilization. This last-mentioned factor, which is called the heat increment, is measured as the increase of heat production which is incident to the entire process of nutrition.

The heat increment is an exceedingly complex phenomenon, and as related to feeds for cattle has not been quantitatively analyzed; nor has the method of measuring heat increments been thoroughly established. It is desirable, therefore, that the details of this estimation, including base values and other necessary points of reference, should be rigidly standardized.

Base values of heat production may be defined with reference to the curve (established at this institute [2]) which expresses the relation of the heat production to the plane of nutrition. This curve is shown in Figure 1, and in relation thereto the following points of reference in regard to heat production will be discussed: (1) Armsby's [3] computed maintenance requirement of net energy; (2) the basal metabolism, in the usual sense; (3) the hypothetical minimum heat production; [4] (4) the heat production of true fast; (5) the heat production of maintenance; and (6) the heat production of twice maintenance.

Armsby [5] computed the maintenance requirement of net energy by relating the difference in heat at two planes of nutrition to the difference in food, thus deriving a heat increment per unit of food from which he computed the heat production at the point of zero food intake, that is, fast; this process being equivalent to projecting the chord of a segment of the curve of heat production to the point of zero food intake, and implying that the curve of heat production is a straight line.

The values for net energy requirement of maintenance, or fasting katabolism, thus derived were naturally exceedingly variable, since they were determined as from different segments of the curve of heat

[1] Received for publication June 17, 1931; issued January, 1932.

[2] FORBES, E. B., BRAMAN, W. W., KRISS, M., with the collaboration of JEFFRIES, C. D., SWIFT, R. W., FRENCH, R. B., MILLER, R. C., and SMYTHE, C. V. THE ENERGY METABOLISM OF CATTLE IN RELATION TO THE PLANE OF NUTRITION. Jour. Agr. Research 37: 253-300, illus. 1928.

——— BRAMAN, W. W., KRISS, M., with the collaboration of SWIFT, R. W., FRENCH, R. B., SMYTHE, C. V., WILLIAMS, P. S., and WILLIAMS, H. H. FURTHER STUDIES OF THE ENERGY METABOLISM OF CATTLE IN RELATION TO THE PLANE OF NUTRITION. Jour. Agr. Research 40: 37-78, illus. 1930.

[3] ARMSBY, H. P. THE MAINTENANCE RATIONS OF FARM ANIMALS. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 143, 110 p., illus. 1912.

[4] FORBES, E. B., BRAMAN, W. W., KRISS, M., with the collaboration of SWIFT, R. W., FRENCH, R. B., SMYTHE, C. V., WILLIAMS, P. S., and WILLIAMS, H. H. Op. cit.

[5] ARMSBY, H. P. Op. cit.

production; and in the nature of the case they must be lower than the heat production of true fast. Inasmuch as the relation of the heat production to the quantity of feed eaten is such as to be represented by a curve, it is obviously impossible to compute a satisfactory base value by this method.

The so-called "basal metabolism" in the usual sense of signifying the resting heat production in an early post-absorptive status, 12 to 18 hours after the last meal, can not be satisfactorily measured with cattle because of the peculiar physiology of the ruminant alimentary tract, some of the characteristics of which are the following: The long-drawn-out process of digestion of the fibrous feed which cattle eat; the habit of rumination, which contributes an undulatory factor in the energy expense of food utilization; the extensive heat production of fermentation in the rumen; the widely varying content of feed in the alimentary tract as affected by the previous plane of

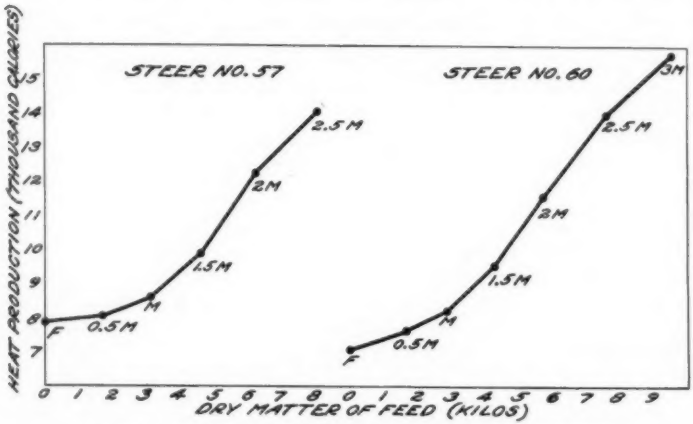

FIGURE 1.—Heat production as affected by the plane of nutrition of cattle

nutrition; and the fact that the rumen of cattle does not completely empty itself, as does the stomach of a human being, but passes on its contents in part as an overflow function. The post-resorptive status, therefore, which is considered to be reached in human beings within 12 to 18 hours after food is taken, seems not to be reached in cattle until the attainment of a state of true fast; and the determination of a "basal metabolism" of cattle, which would be equivalent to the basal metabolism of human beings, seems to the writers to be impracticable.

The ideal base value from which to measure energy metabolism in cattle would be that which Forbes and his associates have described[6] as "the theoretical, minimum, base value of energy metabolism," that is, the energy requirement of an animal living entirely on its own tissues, at 100 per cent efficiency of transformation of their metabolizable energy into vital energy; the heat production representing the actual energy need; there being complete oxidation of metabolizable

[6] FORBES, E. B., BRAMAN, W. W., KRISS, M., with the collaboration of SWIFT, R. W., FRENCH, R. B., SMYTHE, C. V., WILLIAMS, P. S., and WILLIAMS, H. H. Op. cit., p. 53, 56.

nutrients, with no waste heat of transformation, and no stimulation of heat production by metabolites; the organism existing, therefore, in a manner comparable to that of an internal combustion engine operating under such hypothetical conditions that the work done would be equivalent to the potential energy of the fuel. But inasmuch as this value is not measurable, in the present state of knowledge, it is worthy only of definition—for the light which the conception throws upon measurable values.

A base value of heat production to serve in the derivation of heat-increment values for maintenance should be determined under the same conditions, other than as relating to the plane of nutrition, as prevail during the measurements at the upper point of reference, that is, as in feeding practice. Also, in consideration of the character of the component factors of the heat production of cattle, it is obvious that no short-time period of observation can be representative of a day's heat production.

For these reasons it seems to the writers that the most satisfactory measure of the heat production of cattle, for comparative purposes as in the determination of heat increments, is the heat production of a 24-hour day in which the aggregates of time spent in the standing and the lying positions, respectively, are separately determined, and the increased energy expenditure of standing as compared with lying is determined, so that the 24-hour heat production can be computed to a standard day which is uniform as to the proportions of the time spent in these two positions.

The possible employment of the metabolism in the lying position as a base value suggests itself; but is not advocated at this time, since it seems that this would give an imperfectly representative short-time observation. The possible usefulness of this standard, for some purposes, however, will be given consideration.

It is also the belief of the writers that an appropriate physiological measure, rather than elapsed time since feeding, must mark the point of beginning of the measurement of the heat production of fast.

It is the present practice at this institute, therefore, to measure the base value of energy metabolism as the heat production of the first 24 hours after the attainment of the nonprotein respiratory quotient of fat, following an established status of nutrition on a level of protein and energy equilibrium, and computed to the standard day as to standing and lying.

Heat increments for production are naturally measured from the heat production of maintenance, or energy equilibrium, as the lower value; and the writers have suggested the heat production of twice maintenance [7] as a significant, conventional upper value.

The experiments discussed in this paper, however, have to do only with the evaluation of the heat production of fast, and their objective was the standardization of this measurement as the base value for determining heat increments for maintenance.

The use of the respiratory quotient of fat as the criterion for judgment as to the time for beginning this measurement directed especial attention to this quotient, and in this connection it was viewed in the light of the following observations:

[7] FORBES, E. B., BRAMAN, W. W., KRISS, M., with the collaboration of SWIFT, R. W., FRENCH, R. B., SMYTHE, C. V., WILLIAMS, P. S., and WILLIAMS, H. H. Op. cit., p. 65.

In a normal state of nutrition heat is produced by the animal mainly at the expense of the carbohydrate, the fat, and the protein of the food; but a small part of the total is produced at all times from the oxidation of the protein waste from body tissue.

Under such circumstances the respiratory quotient as determined for a cow would be the resultant of the combination of the individual respiratory quotients of the carbohydrate, the protein, and the fat of the food, and of the body loss of protein, as if these were completely oxidized, but modified on the following accounts: (1) The incomplete oxidation of protein katabolized, (2) escape from oxidation of food protein, fat, or carbohydrate, through storage in the body, or through elimination in milk, (3) partial reduction of the carbon of carbohydrate through synthesis into fat, (4) the metabolism of the flora of the alimentary tract, and (5) the elimination of carbon in the form of alkali carbonates formed in the body through the oxidation of organic acid salts.

During fast, however, this picture is greatly simplified. Under conditions of fast the carbohydrate store of the body is virtually exhausted; the organism lives on protein and fat; and the metabolism of the alimentary flora has diminished to negligible proportions. Under these conditions the nonprotein respiratory quotient of fasting cattle is approximately 0.707, as in the other vertebrates which are more frequently studied.

EXPERIMENTAL SUBJECTS

The two steers used as subjects were Shorthorns of unknown history, except that they came from the Pittsburgh market and were originally from West Virginia. Steer No. 17 was between 17 and 21 months, and steer No. 85 between 19 and 22 months of age during these experiments.

It is unfortunately true that the steers were not at all times in perfectly normal condition. At the time of this investigation hemorrhagic septicaemia was present among cattle in a near-by barn, and it is possible that these steers passed through a mild attack of the disease. Also, the slaughter of the steers revealed the presence of pieces of baling wire in the paunch of steer No. 17, and one piece of wire had effected a slight injury to the liver. Inasmuch as the results of the season's metabolism experiments were generally consistent, however, the results are offered for publication.

PLAN OF EXPERIMENTS

A series of seven fasting experiments was conducted with two steers during a 4½-month period. One steer (No. 17) was subjected to fasts four times, and the other steer (No. 85) three times, at intervals varying in length from about 4 to 6 weeks, the fasting periods being of 3 to 6 days' duration. Each fasting period was preceded by a preliminary feeding period of at least 9 days' duration during which the animal was kept on a plane of energy equilibrium.

The ration fed during this preliminary period consisted of alfalfa hay and corn meal (1:1) for steer No. 85, and of corn meal alone for steer No. 17. No physic was given at the end of the preliminary feeding period as was done in some previous fasting studies at this institute.

Between the end of one fasting period and the beginning of the preliminary period of the subsequent fast the animal was allowed a ration of alfalfa hay and corn meal (1:1) equal to twice its maintenance requirement in order to permit the rebuilding of lost tissue.

During the last two to five days of the fasting periods the animals were kept in the respiration calorimeter for continuous heat measurements and for the determination of the respiratory exchange.

The animals were given water during the fasting period, but no salt or other mineral nutriment. At the end of the experimental program the steers were killed, the contents of the alimentary tract were removed and weighed, and the area of the hide was measured.

The outline of experimentation is presented in Table 1.

TABLE 1.—*Schedule of experimentation, daily rations, and live weights of animals*

Period No.	Steer No.	Preliminary feeding period on experimental rations	Rations fed during preliminary period		Fasting periods[a]	Calorimeter period	Interval between time of last feeding and start of calorimeter period	Live weights of animals—	
			Alfalfa hay	Corn meal				On entering calorimeter	On leaving calorimeter
		Days	*Kgm.*	*Kgm.*	*Days*	*Days*	*Hours*	*Kgm.*	*Kgm.*
7	17	23	----------	2.514	6	5	36	333.0	315.2
8	85	9½	1.614	1.680	6	4	60	341.3	330.3
9	17	10	----------	2.486	6	4	60	328.2	316.8
10	85	9½	1.646	1.712	4	2	60	344.5	335.3
11	17	9½	----------	2.508	5	3	60	340.0	335.6
12	85	10½	1.650	1.716	3	3	12	354.9	340.9
13	17	9½	----------	2.624	4	3	36	354.5	342.5

[a] The fasting period is reckoned from the time the animal missed the first meal to the end of the calorimeter period.

METHODS OF EXPERIMENTATION

The heat production of the animals was measured by direct calorimetry, as usual at this institute, and the carbon dioxide, water vapor, and methane were determined gravimetrically in continuous air samples. In addition, samples of the outcoming air were taken during the calorimetric periods, at intervals of six hours, and were analyzed in duplicate for carbon dioxide and oxygen by means of a Carpenter[8] gas analysis apparatus.

The technic followed in the analysis of the air samples of the first three periods (Nos. 7, 8, and 9) was essentially that of Carpenter.[8] In the last four periods (Nos. 10, 11, 12, and 13) this procedure was advantageously modified. This modification will be discussed later in connection with the respiratory quotients.

The rate of ventilation of the calorimeter was 450 liters per minute during the first fasting period (No. 7), but this rate was reduced by about half in the subsequent periods. This change was found desirable as a means of increasing the difference in composition of the incoming and the outgoing air, thus favoring accuracy in the determination of respiratory quotients. It does not appear that this change in ventilation affected the rate of heat production.

In order to obtain an accurate record of the urinary excretion in relation to time, for the proper accounting of the protein metabolism,

[8] CARPENTER, T. M. AN APPARATUS FOR THE EXACT ANALYSIS OF AIR IN METABOLISM INVESTIGATIONS WITH RESPIRATORY EXCHANGE CHAMBERS. Jour. Metabolic Research 4: 1–25, illus. 1923.

the exact time of each voiding of urine was recorded. This was made possible by a special device constructed for the purpose. The urine box was placed on one end of a small rocking platform which was supported by a rod in the middle and balanced by weights on the end opposite the urine receptacle. When the urine was voided its weight caused the box to descend and to make an electrical contact which caused the ringing of a bell. The urine was then removed and weighed.

Another new feature in these experiments was employed in the determination of the difference in heat production between standing and lying. In order to permit the measurement of heat and carbon dioxide during the standing position for a considerable length of time each animal was forced to stand for about three and a half hours by means of a chain passing beneath its body, from side to side of the calorimeter stall. This was done with steer No. 17 in subperiod 8 of period 9, and with steer No. 85 in subperiod 7 of period 8.

EXPERIMENTAL DATA

The results of the gravimetric analysis of the air for 12-hour subperiods are presented in Table 2. These include data for carbon dioxide, water vapor, and methane production by the experimental animals during each calorimeter day.

TABLE 2.—*Carbon dioxide, water vapor, and methane eliminated per day, during fast*

Steer No.	Period No.	Subperiod No. and day of fast	Elimination of—		
			Carbon dioxide	Water	Methane
			Grams	*Grams*	*Grams*
		Subperiod 1	899.0	1,199.6	3.95
		Subperiod 2	883.4	1,138.0	3.33
		Second day	1,782.4	2,337.6	7.28
		Subperiod 3	870.8	1,114.8	1.97
		Subperiod 4	874.8	1,145.7	1.85
		Third day	1,745.6	2,260.5	3.82
		Subperiod 5	892.6	1,139.7	1.88
17	7	Subperiod 6	828.1	1,041.7	1.17
		Fourth day	1,720.7	2,181.4	3.05
		Subperiod 7	830.3	1,070.4	0.0
		Subperiod 8	773.3	943.2	.70
		Fifth day	1,603.6	2,013.6	.70
		Subperiod 9	772.5	975.1	.71
		Subperiod 10	767.2	960.1	.95
		Sixth day	1,539.7	1,935.2	1.66
		Subperiod 1	967.7	1,165.0	1.89
		Subperiod 2	973.7	1,167.5	1.92
		Third day	1,941.4	2,332.5	3.81
		Subperiod 3	943.1	1,109.5	1.37
		Subperiod 4	946.7	1,150.7	1.61
		Fourth day	1,889.8	2,260.2	2.98
85	8	Subperiod 5	932.0	1,152.9	1.43
		Subperiod 6	903.9	1,124.3	1.22
		Fifth day	1,835.9	2,277.2	2.65
		Subperiod 7	916.4	1,134.5	1.08
		Subperiod 8	902.4	1,169.1	.75
		Sixth day	1,818.8	2,303.6	1.83

TABLE 2.—*Carbon dioxide, water vapor, and methane eliminated per day, during fast*—Continued

Steer No.	Period No.	Subperiod No. and day of fast	Elimination of—		
			Carbon dioxide	Water vapor	Methane
			Grams	*Grams*	*Grams*
		Subperiod 1	950.4	1,196.7	1.63
		Subperiod 2	899.9	1,208.0	1.31
		Third day	1,850.3	2,404.7	2.94
		Subperiod 3	896.3	1,204.0	1.22
		Subperiod 4	908.9	1,241.1	1.24
		Fourth day	1,805.2	2,445.1	2.46
17	9	Subperiod 5	882.0	1,161.1	
		Subperiod 6	837.5	1,153.2	1.23
		Fifth day	1,719.5	2,314.3	1.23
		Subperiod 7	865.3	1,246.5	1.37
		Subperiod 8	816.8	1,164.5	
		Sixth day	1,682.1	2,411.0	1.37
		Subperiod 1	1,018.4	1,192.1	1.44
		Subperiod 2	962.9	1,147.9	1.45
		Third day	1,981.3	2,340.0	2.89
85	10	Subperiod 3	958.8	1,082.5	1.19
		Subperiod 4	952.8	1,096.1	.95
		Fourth day	1,911.6	2,178.6	2.14
		Subperiod 1	1,026.1	1,222.5	2.61
		Subperiod 2	1,034.5	1,239.3	1.10
		Third day	2,060.6	2,461.8	3.71
		Subperiod 3	972.8	1,126.3	.87
17	11	Subperiod 4	922.1	1,043.0	.79
		Fourth day	1,894.9	2,169.3	1.66
		Subperiod 5	928.9	1,047.8	1.24
		Subperiod 6	898.6	987.4	.96
		Fifth day	1,827.5	2,035.2	2.20
		Subperiod 1	1,278.9	1,249.6	20.68
		Subperiod 2	1,150.0	1,295.8	7.98
		First day	2,428.9	2,545.4	28.66
		Subperiod 3	1,023.0	1,245.4	5.24
85	12	Subperiod 4	1,018.9	1,208.6	3.10
		Second day	2041.9,	2,454.0	8.34
		Subperiod 5	984.1	1,161.9	2.46
		Subperiod 6	963.3	1,239.8	2.14
		Third day	1,947.4	2,401.7	4.60
		Subperiod 1	1,103.2	1,237.7	3.47
		Subperiod 2	1,031.3	1,160.5	2.31
		Second day	2,134.5	2,398.2	5.78
		Subperiod 3	1,044.0	1,140.8	1.72
17	13	Sub period 4	1,022.4	1,121.2	1.60
		Third day	2,066.4	2,262.0	3.32
		Subperiod 5	1,043.7	1,153.3	1.43
		Subperiod 6	983.5	1,093.6	1.25
		Fourth day	2,027.2	2,246.9	2.68

The carbon dioxide production shows a gradual decline with the progress of the fast, which is in harmony with previous observations.

The figures for methane show that on the third day of fast the fermentation was very slight in extent, but as the fast continued the quantity of methane was still further reduced.

The data for water vapor are utilized in the computation of the latent heat of water vaporization, which forms a part of the total heat emission.

TABLE 3.—*Heat emission and heat production per day, during fast*

Steer No.	Period No.	Sub-period No.	Calorimeter temperature	Water consumption	Heat emission			Correction for body gain	Heat production
					By radiation and conduction	As latent heat of water vapor	Total		
			°C.	Kgm.	Calories	Calories	Calories	Calories	Calories
		1	17.8	5.14	2,157.1	704.1	2,861.2	+19.4	2,880.6
		2	17.8		2,153.7	668.0	2,821.7	−56.6	2,765.1
		3	17.8	6.51	2,165.5	654.4	2,819.9	+62.6	2,882.5
		4	17.8		2,216.2	672.5	2,888.7	−65.3	2,823.4
17	7	5	17.7	.91	2,208.8	671.6	2,970.4	−42.5	2,927.9
		6	17.8		2,078.3	611.5	2,689.8	−45.3	2,644.5
		7	17.7	1.90	2,199.9	631.3	2,831.2	−30.3	2,800.9
		8	17.8		1,973.0	553.6	2,526.6	−43.8	2,482.6
		9	17.8	2.42	1,932.1	572.4	2,504.5	−6.6	2,497.9
		10	17.8		2,001.4	563.6	2,565.0	−59.6	2,505.4
		1	17.6	3.22	2,318.0	683.9	3,001.9	+10.9	3,012.8
		2	17.6		2,524.4	685.3	3,209.7	−46.5	3,163.2
		3	17.6	6.53	2,377.6	651.3	3,028.9	+81.5	3,110.4
85	8	4	17.6		2,425.0	675.4	3,100.4	−62.3	3,038.1
		5	17.6	3.71	2,315.2	676.7	2,991.9	+30.5	3,022.4
		6	17.7		2,335.3	660.0	2,995.3	−53.6	2,941.7
		7	17.7	2.76	2,420.9	666.0	3,086.9	+0.8	3,087.7
		8	17.7		2,271.4	686.3	2,957.7	−59.2	2,898.5
		1	17.8	8.38	2,281.7	702.5	2,984.2	+130.8	3,115.0
		2	17.7		2,272.8	709.1	2,981.9	−50.6	2,931.3
		3	17.7	3.44	2,239.1	706.8	2,945.9	−17.9	2,928.0
17	9	4	17.7		2,288.7	728.5	3,017.2	−64.3	2,952.9
		5	17.7	3.52	2,218.6	681.5	2,900.1	+1.5	2,901.6
		6	17.7		2,129.4	676.6	2,806.0	−60.7	2,745.3
		7	17.8	1.60	2,068.0	731.7	2,799.7	−27.9	2,771.8
		8	17.8		2,050.4	683.6	2,734.0	−35.4	2,698.6
		1	17.6	2.26	2,629.2	699.7	3,328.9	−17.6	3,311.3
85	10	2	17.7		2,447.1	673.8	3,120.9	−64.2	3,056.7
		3	17.7	4.76	2,508.3	635.4	3,143.7	+44.5	3,188.2
		4	17.6		2,480.6	643.4	3,124.0	−66.8	3,057.2
		1	17.6	4.90	2,590.9	717.6	3,308.5	+51.7	3,360.2
		2	17.5		2,697.0	727.5	3,424.5	−44.2	3,380.3
17	11	3	17.6	6.50	2,438.4	661.1	3,099.5	+63.7	3,163.2
		4	17.7		2,394.4	612.2	3,006.6	−36.3	2,970.3
		5	17.7	4.95	2,455.6	615.1	3,070.7	+34.7	3,105.4
		6	17.7		2,387.9	579.6	2,967.5	−73.2	2,894.3
		1	17.7	5.50	2,928.8	733.5	3,662.3	+41.9	3,704.2
		2	17.7		2,803.0	760.6	3,563.6	−61.3	3,502.3
85	12	3	17.7	5.24	2,456.1	731.1	3,187.2	+57.8	3,245.0
		4	17.7		2,541.6	709.4	3,251.0	−46.2	3,204.8
		5	17.7	.45	2,561.5	682.0	3,243.5	−43.8	3,199.7
		6	17.7		2,457.2	727.8	3,185.0	−43.1	3,141.9
		1	17.7	5.77	2,854.6	726.5	3,581.1	+44.9	3,626.0
		2	17.6		2,715.0	681.2	3,396.2	−49.3	3,346.9
17	13	3	17.6	1.33	2,774.0	669.6	3,443.6	−30.6	3,413.0
		4	17.6		2,654.4	658.1	3,312.5	−33.7	3,278.8
		5	17.6	1.52	2,769.3	677.0	3,446.3	−26.8	3,419.5
		6	17.6		2,605.4	642.0	3,247.4	−54.4	3,193.0

In Table 3 are recorded the data for heat emission and heat production. Records of the calorimeter temperatures and of the water consumption are included, as these have a bearing on the heat measurements. The practical constancy of the calorimeter temperatures is an index of the efficiency with which the conditions in the calorimeter

were controlled for the most accurate measurement of the heat emission. The data for water consumption enter into the computation of the heat correction for body gain. The animals were offered water only once a day, that is, during every other subperiod. The quantities of water consumed were not large, and no definite influence of water consumption on the metabolism is apparent in these experiments.

The figures for heat production presented in Table 3 are uncorrected for differences in time spent by the animal in the standing position, or for differences in live weight, and on this account are not strictly comparable. For comparative purposes the daily heat production of the experimental animals was corrected according to the customary procedure, to a standard day of 12 hours standing and 12 hours lying; and the corrected values per head, per 100 kgm. live weight, and per square meter of body surface, are set forth in Table 4, together with the corresponding nonprotein respiratory quotients, and rate of ventilation of the calorimeter. The difference in heat production per hour, while standing, as compared with that while lying, was found to be 45.5 calories for steer No. 17, and 65.6 calories for steer No. 85. These figures form the basis of the corrections of the heat production to the standard day in these experiments. The measured areas of the hides as given in Table 6 were used as the basis for the computation of the heat production per square meter of body surface.

TABLE 4.—*Heat production of fasting steers, corrected to the standard day*

Animal and period number and day of fast	Date	Time spent standing	Heat production corrected to standard day			Respiratory quotient (nonprotein) [a]	Rate of ventilation per minute
			Per head	Per 100 kgm. live weight	Per square meter of body surface		
Steer No. 17,[b] period 7:		*Hours*	*Calories*	*Calories*	*Calories*		*Liters*
Second day	Dec. 31-Jan. 1	8.0	5,828	1,798	1,239	(0.72)	449
Third day	Jan. 1-2	5.3	6,011	1,855	1,278	(.69)	443
Fourth day	Jan. 2-3	8.6	5,727	1,767	1,217	(.70)	451
Fifth day	Jan. 3-4	8.0	5,466	1,687	1,162	(.70)	456
Sixth day	Jan. 4-5	5.4	5,303	1,636	1,127	(.71)	455
Steer No. 17,[b] period 9:							
Third day	Jan. 29-30	7.3	6,260	1,941	1,330	(.73)	211
Fourth day	Jan. 30-31	6.8	6,108	1,894	1,298	(.68)	211
Fifth day	Jan. 31-Feb. 1	6.8	5,884	1,824	1,251	(.70)	210
Sixth day	Feb. 1-2	10.3	5,547	1,720	1,179	.....	382
Steer No. 17,[b] period 11:							
Third day	Mar. 13-14	13.2	6,686	1,979	1,421	.74	211
Fourth day	Mar. 14-15	6.7	6,375	1,887	1,355	.71	211
Fifth day	Mar. 15-16	6.6	6,246	1,849	1,328	.71	209
Steer No. 17,[b] period 13:							
Second day	Apr. 23-24	9.2	7,100	2,037	1,509	.71	212
Third day	Apr. 24-25	7.8	6,883	1,975	1,463	.72	211
Fourth day	Apr. 25-26	9.1	6,745	1,935	1,434	.71	206
Steer No. 85,[b] period 8:							
Third day	Feb. 19-20	8.1	6,432	1,915	1,437	.....	209
Fourth day	Feb. 20-21	6.4	6,516	1,940	1,456	.....	211
Fifth day	Feb. 21-22	6.0	6,358	1,893	1,421	.....	209
Sixth day	Feb. 22-23	10.1	6,111	1,820	1,366	.....	211
Steer No. 85,[b] period 10:							
Third day	Apr. 2-3	8.3	6,611	1,945	1,477	.71	213
Fourth day	Apr. 3-4	8.4	6,481	1,907	1,448	.71	212
Steer No. 85,[b] period 12:							
First day	May 13-14	7.3	7,515	2,160	1,679	.81	213
Second day	May 14-15	7.4	6,752	1,941	1,509	.71	212
Third day	May 15-16	7.1	6,663	1,915	1,489	.70	210

[a] Results inclosed in parentheses contain an element of error.
[b] The correction for standing per hour for steer No. 17 is 45.5 calories, and for steer No. 85, 65.6 calories.

It will be observed that the heat production of fast generally declined with the progress of fast, and that it did not reach a constant value. This was true with both animals. Steer No. 17 was fed a maintenance ration of corn meal alone during the preliminary periods with the expectation that by this means the animal might be brought to a state of true fast in a shorter time of fasting than would be required for steer No. 85, which received roughage in addition to

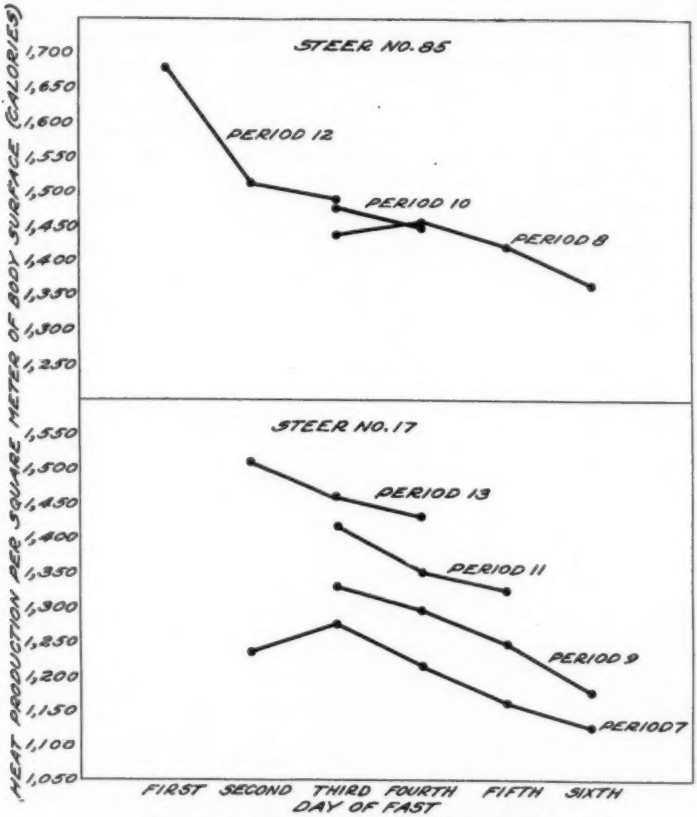

FIGURE 2.—Daily heat production of steers in repeated fasting trials

the corn prior to the fast. A comparison of the respiratory quotients does not indicate that such a result was attained. Thus a respiratory quotient of 0.71 (representing, essentially, oxidation of fat) was reached on the second day of fast, with steer No. 85, period 12, as well as with steer No. 17, period 13. On the third day of fast in period 11 a respiratory quotient of 0.74 was obtained with steer No. 17, and the indication is that in this period the animal did not reach a true fasting status until the fourth day of fast.

In the course of these experiments it was found that if samples of air are stored (in glass tubes) for a considerable length of time (a week or

more) the composition changes. On this account the air analyses, and therefore the respiratory quotients, for periods 7 and 9 contain an element of error, and in Table 4 are inclosed in parentheses. In periods 10, 11, 12, and 13 the air samples were analyzed soon after they were collected, and satisfactory results were obtained.

It was also found more advantageous in the use of Carpenter's gas analysis apparatus to determine the absorption of CO_2 and O_2 together in one aliquot, and the CO_2 content alone in a second aliquot, thus permitting the determination of O_2 by subtraction, than to follow the usual routine in which the O_2 is determined in the same aliquot as that in which the CO_2 is determined, but after the CO_2 is absorbed. The new routine was followed in periods 10, 11, 12, and 13 with much success.

The respiratory quotients reached a constant representing katabolism of fat in these experiments between the second and fourth days of fast. It is noteworthy that the values for heat production per square meter of body surface as reported by Benedict and Ritzman[9], in the same three consecutive 24-hour periods of fast, are considerably higher than those given in Table 4. Benedict and Ritzman's values range from 1,620 to 2,080 calories per day, while those here reported range from 1,217 to 1,509 calories per day, from the second to the fourth days of fast. This difference could not have been due to differences in time spent in the standing position, for in most instances these differences were very slight, that is, the number of hours standing per day was not far from 12. Nor can the temperature of the chamber account for this difference in heat production, for the differences in temperature were also small. It seems most likely that this marked difference is the result of difference in the previous planes of nutrition.

TABLE 5.—*Contents of alimentary tract of steers after fast*

KILOGRAMS DRY MATTER

Animal No. and period of fasting	Contents of—					
	Paunch and reticulum	Omasum	Abomasum	Small intestine	Large intestine	Total alimentary tract
Steer 17, after 4 days' fast	0.628	0.256	0.031	0.122	0.119	1.156
Steer 85, after 3 days' fast	.630	.300	.052	.130	.206	1.318
KILOGRAMS FRESH SUBSTANCE						
Steer 17, after 4 days' fast	24.700	1.752	0.243	3.269	2.364	32.328
Steer 85, after 3 days' fast	29.300	2.189	.452	3.615	3.214	38.770

Attention is called to the facts (Table 4 and fig. 2) that the curves of heat production of steer No. 17 were nearly parallel and that the values increased in each successive period of fast. Thus, on the third day of fast in periods 7, 9, 11, and 13 the heat production per 100 kgm. live weight was 1,855, 1,941, 1,979, and 1,975 calories, respectively; and per square meter of body surface was 1,278, 1,330, 1,421, and 1,463 calories, respectively. The experimental evidence throws no light on the cause of this increasing heat production during repeated

[9] BENEDICT, F. G., and RITZMAN, E. G. THE METABOLISM OF THE FASTING STEER. 246 p., illus. Washington, D. C. 1927. (Carnegie Inst. Wash. Pub. 377.)

fast. This condition did not prevail with steer No. 85. In this connection it is necessary to consider the facts noted on page 1006 as to slight abnormalities in the physical condition of the animal subjects.

The contents of the alimentary tracts of the steers after fasting, and measurements of their surface areas are shown in Tables 5 and 6.

TABLE 6.—*Measurements of surface area of steers after fast*

Animal No.	Live weight of animal	Measured area of hide	Computed by Moulton's formula	Computed by Hogan's formula
	Kilograms	*Square meters*	*Square meters*	*Square meters*
Steer 17	343	4.705	4.552	3.999
Steer 85	341	4.475	4.539	4.091

The urinary nitrogen excreted per 12-hour subperiod during fast was as follows:

	Grams
Steer 17, period 7	18.3
Steer 17, period 9	18.6
Steer 17, period 11	21.3
Steer 17, period 13	19.4
Steer 85, period 8	19.4
Steer 85, period 10	21.4
Steer 85, period 12	19.9

SUMMARY

A series of experiments was conducted as a contribution to the standardization of the determination of the heat production of fasting cattle.

Two steers were subjected to 3 and 4 fasts, respectively, of 3 to 6 days' duration, at intervals of 4 to 6 weeks—in all cases following preparatory periods of feeding on a plane of energy equilibrium.

The heat production was measured by direct calorimetry, checked by determination of the heat by the respiratory-quotient procedure, during the last two to five days of the several fasting periods.

The heat production generally diminished continuously as the fast progressed, and no definite constant level of heat production was reached.

If the routine measurement of the heat production of fast is arbitrarily standardized—as the writers propose—as the heat production of the first 24 hours after the attainment of the nonprotein respiratory quotient of fat, following an established status of nutrition on a level of protein and energy equilibrium, it seems that true fast may be attained as early as the second day after the withdrawal of food, and that it will certainly be reached by the fourth day.

New minor points of procedure which were employed in this study were (1) the use of an apparatus providing for recording the exact times of urination, and the separate collection of each portion of urine passed, as a basis for the proper accounting for the protein metabolism; (2) the enforced standing of the experimental subject, for several hours, to give improved data providing for the computation of the heat production to standard days of 12 hours standing and 12 hours in the lying position; and (3) an improved technic of air analysis by means of the Carpenter apparatus.

THE METABOLIZABLE ENERGY AND NET ENERGY VALUES OF CORN MEAL WHEN FED EXCLUSIVELY AND IN COMBINATION WITH ALFALFA HAY[1]

By E. B. FORBES, *Director*, WINFRED W. BRAMAN, MAX KRISS, and R. W. SWIFT, *Associates* in *Animal Nutrition*, with the collaboration of R. C. MILLER, R. B. FRENCH, T. V. LETONOFF, and G. R. SHARPLESS, *Institute of Animal Nutrition, Pennsylvania State College*

INTRODUCTION

The purpose of this investigation, which was conducted in the fall of 1929, was to determine whether the energy value of a ration is equal to the sum of the separately determined energy values of its components; or, in other words, whether the energy value of a feeding stuff is affected by the combination in which it is fed with other feeding stuffs.

So far as the writers are aware, all existing values for metabolizable energy and net energy of concentrates for herbivorous animals have been determined indirectly, that is, by difference between such values for mixed rations (roughage plus concentrate) and for rations of roughage alone, this procedure involving the assumption that the nutritive value of the roughage in the mixed ration is the same as when it is fed alone. It is important, therefore, to learn the extent of any such error as may result from this indirect procedure, and to determine the possibility of more direct measurements of energy values.

The only directly determined values of the digestibility of a concentrate, of which the writers are aware, are those of Beach [2] on the digestibility of corn meal. Beach fed two dry, farrow cows exclusively on corn meal for a period of 130 days, and determined the digestibility of the meal from the feces collected during the last week of this interval. In other periods with the same animals he also determined the digestibility of hay fed by itself. The main conclusion drawn from these experiments was that the digestible nutrients of the corn meal were utilized by the animals more efficiently than the digestible nutrients of the hay, and that the exclusive feeding of meal produced no unfavorable effects.

The data of Beach on the digestibility of corn meal are of especial interest since they throw light on the question as to whether ruminants can efficiently utilize concentrated feeds without the addition of roughages. They did not, however, provide a basis for direct comparison of the utilization of corn meal when fed alone and when fed in combination with roughage. Such a comparison was made possible in the present experiments.

The experimental subjects were the same two steers, Nos. 17 and 85, that were used in the preceding fasting study [3], and were approximately fifteen to seventeen months old at the time of these experiments.

---

[1] Received for publication June 17, 1931; issued January, 1932.

[2] BEACH, C. L. THE FACILITY OF DIGESTION OF FOODS A FACTOR IN FEEDING. Conn. (Storrs) Agr. Expt. Sta. Bul. 43, 23 p. 1906.

[3] FORBES, E. B., BRAMAN, W. W., KRISS, M., and SWIFT, R. W., with the collaboration of MILLER, R. C., FRENCH, R. B., LETONOFF, T. V., and SHARPLESS, G. R. THE FASTING METABOLISM OF CATTLE AS A BASE VALUE OF HEAT PRODUCTION IN THE DETERMINATION OF THE NET ENERGY OF FEEDING STUFFS Jour. Agr. Research (1931) 43: 1003-1014, illus. 1932.

PLAN AND METHOD OF EXPERIMENTATION

The schedule of experimentation is presented in Table 1.

TABLE 1.—*Schedule of experimentation, daily rations, and live weights of animals*

Period No.	Steer No.	Preliminary feeding period on experimental rations	Total digestion period	Calorimeter period	Rations fed daily		Average live weight of animal
					Alfalfa hay	Corn meal	
					Kgm.	*Kgm.*	*Kgm.*
1	17	Sept. 14–24	Sept. 25–Oct. 12	Oct. 9–12	5.374		364.2
3	17	Oct. 12–22	Oct. 23–Nov. 9	Nov. 6–9		2.514	327.6
4	85	Nov. 23–Dec. 3	Dec. 4–21	Dec. 18–21	5.142		332.1
5	17	Nov. 12–19	Nov. 20–Dec. 7	Dec. 4–7	1.664	1.728	338.3

Three metabolism experiments were conducted with steer No. 17 and one with steer No. 85. During the first period steer No. 17 received a maintenance ration of alfalfa hay alone. In period 3 this steer received a maintenance ration of corn meal alone, and in period 5 a maintenance ration of alfalfa hay and corn meal, half and half.

Steer No. 85 received a maintenance ration of alfalfa hay in period 4. Period 2 was a metabolism experiment with steer No. 85, also on alfalfa hay alone, but owing to refusal of large quantities of feed this experimental period has been discarded.

With the exception of No. 5 each experimental period was of 28 days' duration, the first 10 days of which constituted the preliminary and the last 18 days the digestion period. The last 3 days of the digestion period constituted the calorimetric period, during which the animal was kept in the respiration calorimeter for the measurement of the heat production and of the gaseous products of metabolism.

EXPERIMENTAL RESULTS

The experimental data are presented in Tables 2 to 13, inclusive.

In Table 2 are given the direct results of the digestion trials on the rations of alfalfa hay, of corn meal, and of mixed alfalfa hay and meal, and also the digestibility of the corn meal as computed by difference, from the results from the mixed ration.

A comparison of the values for digestibility of the alfalfa-hay ration in periods 1 and 4 shows good agreement with respect to all nutrients except ether extract. Apparent inconsistencies in digestion coefficients of ether extract of alfalfa hay have been observed and discussed in reports of other experiments from this institute.[4] The coefficients of digestibility of the various nutrients in the alfalfa hay, and in the mixed ration (period 5) as well, agree satisfactorily with corresponding coefficients obtained on the same feeds in previous studies.

[4] FORBES, E. B., BRAMAN, W. W., and KRISS, M., with the collaboration of JEFFRIES, C. D., SWIFT, R. W., FRENCH, R. B., MILLER, R. C., and SMYTHE, C. V. THE ENERGY METABOLISM OF CATTLE IN RELATION TO THE PLANE OF NUTRITION. Jour. Agr. Research 37: 253–300, illus., 1928.

TABLE 2.—*Digestibility of rations*

Period No.	Steer No.	Item	Dry matter	Organic matter	Crude protein	Crude fiber	Ether extract	Nitrogen-free extract	Carbon	Energy (calories)	Nitrogen
		Salt.....grams	30								
		Alfalfa hay.....do	4,827	4,438.2	744.4	1,703.8	65.7	1,924.3	2,198.1	21,154.9	119.1
1	17	Total feed.....do	4,857	4,438.2	744.4	1,703.8	65.7	1,924.3	2,198.1	21,154.9	119.1
		Feces.....do	1,976	1,788.7	216.9	964.1	57.0	550.7	955.4	9,439.9	34.7
		Digestibility.....per cent	59.3	59.7	70.9	43.4	13.2	71.4	56.5	55.4	70.9
		Salt.....grams	30								
		Alfalfa hay.....do	4,431	4,123.2	744.4	1,620.8	79.8	1,678.2	2,061.1	20,008.7	119.1
4	85	Total feed.....do	4,461	4,123.2	744.4	1,620.8	79.8	1,678.2	2,061.1	20,008.7	119.1
		Feces.....do	1,790	1,588.2	196.9	823.8	75.7	491.8	874.6	8,534.1	31.5
		Digestibility.....per cent	59.9	61.5	73.5	49.2	5.1	70.7	57.6	57.3	73.6
		Salt.....grams	30								
		Alfalfa hay.....do	1,499	1,378.3	231.3	529.1	20.4	597.5	682.6	6,569.5	37.0
5	17	Corn meal.....do	1,495	1,472.2	154.4	38.9	54.0	1,224.9	691.5	6,741.4	24.7
		Total feed.....do	3,024	2,850.5	385.7	568.0	74.4	1,822.4	1,374.1	13,310.9	61.7
		Feces.....do	781	705.4	111.9	337.3	35.6	220.6	383.4	3,775.9	17.9
		Digestibility.....per cent	74.2	75.3	71.0	40.6	52.2	87.9	72.1	71.6	71.0
		Salt.....grams	30								
3 [a] (Average of last 8 days)	17	Corn meal.....do	2,174	2,140.7	222.5	53.9	83.7	1,780.6	1,003.6	9,817.4	35.6
		Feces.....do	305	258.0	48.1	61.8	12.7	135.4	140.9	1,479.8	7.7
		Digestibility.....per cent	86.2	87.9	78.4	(?)	84.8	92.4	86.0	84.9	78.4
		Salt.....grams	30								
3 [a] (Average of last 3 days)	17	Corn meal.....do	2,174	2,140.7	222.5	53.9	83.7	1,780.6	1,003.6	9,817.4	35.6
		Feces.....do	287	246.2	46.9	48.3	8.0	143.0	131.7	1,383.2	7.5
		Digestibility.....per cent	87.0	88.5	78.9	10.4	90.4	92.0	86.9	85.9	78.9
5	17	Percentage digestibility of corn meal [b]	90.6	89.8	71.1	(?)	66.9	95.9	87.5	87.5	71.3

[a] Exclusive corn-meal feeding.

[b] Computed from the mixed rations.

In the corn-meal trial (period 3) there was great irregularity in the excretion of feces from day to day, the amounts excreted during the first 10 days greatly exceeding those excreted during the last 8 days of the digestion trial. It was therefore suspected that the feces collected during the first part of the digestion period might not truly represent the corn meal fed—in other words, that the preliminary period of 10 days might be insufficient as a transition period from exclusive roughage to exclusive concentrate feeding. The daily collections of feces were therefore separately analyzed with the thought that the crude fiber content would show whether the feces were contaminated with hay residues.

TABLE 3.—*Composition of the daily feces of steer No. 17 when on corn meal alone in period 3*

Day of digestion period	Fresh weight of feces	Dry matter		Composition of the dry matter							
				Crude fiber		Ash		Ether extract		Nitrogen	
	Grams	*Per cent*	*Grams*	*Per cent*	*Grams*	*Per cent*	*Grams*	*Per cent*	*Grams*	*Per cent*	*Grams*
First	2,833	26.922	762.7	49.272	375.8						
Second	1,920	21.181	406.7	47.769	194.3						
Third	4,613	17.417	803.4	54.426	437.3						
Fourth	6,339	15.395	975.9	52.772	515.0						
Fifth	3,580	20.694	740.8	47.854	354.5						
Sixth	3,449	22.600	779.5	44.624	347.8						
Seventh	2,592	25.920	671.8	40.016	268.8						
Eighth	1,803	26.018	469.1	37.452	175.7						
Ninth	1,915	24.996	478.7	32.888	157.4						
Tenth	1,534	24.971	383.1	28.882	110.6	13.648	52.3	2.605	10.0	2.119	8.1
Eleventh	261	25.823	67.4	27.840	18.8	13.062	8.8			2.112	1.4
Twelfth	1,109	24.019	266.4	24.363	64.9	16.075	42.8	4.703	12.5	2.418	6.4
Thirteenth	809	21.593	174.7	21.806	38.1	16.254	28.4	5.146	9.0	2.598	4.5
Fourteenth	1,260	22.036	277.7	21.723	60.3	16.517	46.2	4.378	12.2	2.491	6.9
Fifteenth	3,579	22.212	795.0	21.113	167.8	16.228	129.0	3.950	31.4	2.507	19.9
Sixteenth	653	22.508	147.0	18.172	26.7	14.548	21.4	2.964	4.4	2.762	4.1
Seventeenth	863	23.029	198.7	19.828	39.4	12.733	25.3	2.611	5.2	2.461	4.9
Eighteenth	2,150	23.912	514.1	15.321	78.8	14.502	74.6	2.803	14.4	2.622	13.5

From the results of these analyses, which are given in Table 3, it is evident that during the first 10 days of the digestion period the feces collected did not accurately represent the corn meal. The quantities of crude fiber in the feces during the first 10 days were very much greater than in the following 8 days, there being an abrupt decrease between the tenth and the eleventh days. The percentages of crude fiber in feces diminished markedly from the first to the last day of the digestion period. Such a diminution is observed even in the last eight days of the collection period, but the amounts of feces collected during these days are relatively small. From these data relating to crude fiber and from the subsequent complete analysis of the daily feces of the last 8 days of the digestion period it was concluded to disregard the feces of the first 10 days of the collection period in the determination of digestibility of the corn meal.

The digestibility of the corn meal was then computed from the data of period 3 on two bases—(1) the feces of the last eight days of the digestion period, (2) the feces of the last three days of the same digestion period, and (3) for comparison with these directly determined values, the digestibility was determined indirectly, from the mixed ration, by the usual difference procedure.

A comparison of the figures for digestibility of corn meal as computed above reveals the fact that the coefficients obtained in the period of

exclusive meal feeding on the two different bases agree well with each other with respect to all nutrients except crude fiber, and that with the exception of the values for ether extract the coefficients computed from the mixed rations agree fairly well with those obtained directly for the corn meal when fed alone. The data indicate that the digestibility of the crude fiber of corn meal is less than the error of determination. These observations find support in the results obtained by Beach,[5] who found negative digestion coefficients for the crude fiber of corn meal.

In general it may be concluded that the digestibility of corn meal when fed alone is not materially different from its digestibility when fed in combination with alfalfa hay.

TABLE 4.—*Carbon dioxide, water vapor, and methane eliminated per day*

Steer No.	Period No.	Calorimeter day	CO_2	C as CO_2	H_2O	CH_4	C as CH_4
			Grams	*Grams*	*Grams*	*Grams*	*Grams*
17	1	First	3,813.6	1,040.0	4,160.1	113.2	84.7
		Second	3,764.5	1,026.6	3,873.1	108.7	81.3
		Third	3,760.7	1,025.5	3,796.8	113.4	84.9
		Average	3,779.6	1,030.7	3,943.3	111.8	83.6
17	3	First	2,829.9	771.7	2,940.5	48.5	36.3
		Second	2,869.5	782.5	2,955.4	49.7	37.2
		Third	2,764.7	753.9	2,831.7	50.4	37.7
		Average	2,821.4	769.4	2,909.2	49.5	37.1
85	4	First	3,791.5	1,033.9	4,693.9	106.1	79.4
		Second	3,816.0	1,040.6	5,113.5	106.5	79.7
		Third	3,794.7	1,034.8	5,224.5	105.0	78.6
		Average	3,800.7	1,036.4	5,010.6	105.9	79.2
17	5	First	2,953.0	805.3	2,756.5	95.3	71.4
		Second	2,927.4	798.3	2,609.9	89.9	67.3
		Third	3,005.4	819.6	2,748.9	86.5	64.7
		Average	2,961.9	807.7	2,705.1	90.6	67.8

Table 4 presents the daily elimination of carbon dioxide, water vapor, and methane by the experimental animals. Inspection of the data reveals great uniformity in the daily amounts of these gases eliminated during the 3-day calorimeter periods. This situation usually signifies that there were no serious disturbances in the metabolism of the animal and that the data are satisfactory.

TABLE 5.—*Methane production of steer No. 17 as affected by the character of the ration*

Period No.	Kind of food	Digested carbohydrates	Methane	Methane per kilogram of—		Dry matter of food
				Dry matter	Digestible carbohydrates	
		Kgm.	*Grams*	*Grams*	*Grams*	*Kgm.*
1	Alfalfa hay	2.113	112	23.2	53.0	4.827
3	Corn meal	1.637	50	23.0	30.5	2.174
4	Alfalfa hay	1.983	106	23.9	53.5	4.431
5	Mixed ration	1.833	91	30.4	49.6	2.994

[5] BEACH, C. L. Op. cit.

Table 5 shows that the methane production was rather closely in accord with the dry matter but not closely in accord with the digestible carbohydrate of the ration—as is readily understandable since the carbohydrate is not digested in any considerable proportion in the rumen, which is presumably the place of production of the principal part of the methane. The mixed diet of corn and hay seems to have been superior to either component as a medium for the growth of methane-producing organisms. The fact that the methane production per kilogram of dry matter of the mixed ration was greater than from the ration of hay alone may perhaps be due to the possibility that the corn in the mixed ration was more extensively fermented than was the corn fed by itself—the corn in the mixed ration being swallowed into the paunch and subjected to rumination as usual, while the corn fed by itself may have been in part or in whole swallowed past the paunch; at least, it was not subjected to rumination. The very low value for methane from corn, as compared with digestible carbohydrates, tends to support these suggestions.

TABLE 6.—*Balance of matter and energy per day*

PERIOD 1, STEER NO. 17

Item	Dry matter	Water	Nitrogen	Carbon	Energy
Income:	*Grams*	*Grams*	*Grams*	*Grams*	*Calories*
Alfalfa hay	4,827	547	119.1	2,198.1	21,154.9
Water		15,248			
Total	4,827	15,795	119.1	2,198.1	21,154.9
Outgo:					
Feces	1,904	7,274	34.7	955.4	9,439.9
Urine	335	6,360	81.7	129.1	1,108.9
Methane	111.8			83.6	1,491.9
Carbon dioxide	3,779.6			1,030.7	
Water vapor		3,493			
Metabolizable: Income minus urine, feces, and methane					9,114.2
Body balances:					
Fat	−12.0			−9.2	−114.0
Protein	+16.2		+2.7	+8.5	+72.2
Water		−1,782			
Computed heat production					9,156.0
Observed heat production					9,149.3

PERIOD 5, STEER NO. 17

Income:					
Alfalfa hay	1,499	165	37.0	682.6	6,569.5
Corn meal	1,495	233	24.7	691.5	6,741.4
Water		8,337			
Total	2,994	8,735	61.7	1,374.1	13,310.9
Outgo:					
Feces	765	3,414	17.9	383.4	3,775.9
Urine	138	2,626	34.3	63.5	619.9
Methane	90.6			67.8	1,209.0
Carbon dioxide	2,961.9			807.7	
Water vapor		2,705			
Metabolizable: Income minus urine, feces, and methane					7,706.1
Body balances:					
Fat	+28.5			+21.8	+270.8
Protein	+57.0		+9.5	+29.9	+254.1
Water		−10			
Computed heat production					7,181.2
Observed heat production					7,079.6

TABLE 6.—*Balance of matter and energy per day*—Continued

PERIOD 4, STEER NO. 85

Item	Dry matter	Water	Nitrogen	Carbon	Energy
	Grams	*Grams*	*Grams*	*Grams*	*Calories*
Income:					
Alfalfa hay	4,431	711	119.1	2,061.1	20,008.7
Water		17,810			
Total	4,431	18,521	119.1	2,061.1	20,008.7
Outgo:					
Feces	1,832	10,472	31.5	874.6	8,534.1
Urine	256	4,861	81.3	113.1	1,271.6
Methane	105.9			79.2	1,413.1
Carbon dioxide	3,800.7			1,036.4	
Water vapor		5,011			
Metabolizable: Income minus urine, feces, and methane					8,789.9
Body balances:					
Fat	−81.2			−62.1	−771.4
Protein	+37.8		+6.3	+19.9	+168.6
Water		−1,823			
Computed heat production					9,392.7
Observed heat production					9,375.5

PERIOD 3, STEER NO. 17

Income:					
Corn meal	2,174	340	35.6	1,003.6	9,817.4
Water		7,420			
Total	2,174	7,760	35.6	1,003.6	9,817.4
Outgo:					
Feces (average of eight days)	305	935	7.7	140.9	1,479.8
Urine	201	3,825	35.8	47.6	422.4
Methane	49.5			37.1	660.5
Carbon dioxide	2,821.4			769.4	
Water vapor		2,909			
Metabolizable: income minus urine, feces and methane					7,254.7
Body balances:					
Fat	+43.8			+33.5	+416.1
Protein	−47.4		−7.9	−24.9	−211.3
Water		+91			
Computed heat production					7,049.9
Observed heat production					7,024.9

The balances of matter and energy are presented in Table 6. In the corn-meal experiment (period 3) the data for feces represent an average of the last eight days of the digestion period, instead of 18 days as in the other periods. This departure is justified by the apparent lag in the elimination of feces during the feeding of corn alone, as indicated by the prolonged appearance in the feces of crude fiber from the previous period.

TABLE 7.—*Energy of the urine and of the protein, corrected for incomplete oxidation of protein gained or lost*

Period No.	Balance of N	Correction (N×7.45)	Energy of urine		Energy of protein	
			Uncorrected for N equilibrium	Corrected	Uncorrected (grams protein×5.7)	Corrected
	Grams	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*
1	+2.7	+20.1	1,088.8	1,108.9	92.3	72.2
3	−7.9	−58.9	481.3	422.4	270.2	211.3
4	+6.3	+46.9	1,224.7	1,271.6	215.5	168.6
5	+9.5	+70.8	549.1	619.9	324.9	254.1

TABLE 8.—*Heat emission and heat production per day*

Steer No.	Period No.	Calorimeter days	Sub-period No.	Heat emission			Corrections (body gain)	Heat production
				By radiation and conduction	As latent heat of water vapor	Total		
				Calories	*Calories*	*Calories*	*Calories*	*Calories*
		First	1	3,464.0	1,221.0	4,685.0		
			2	3,585.0	1,221.0	4,806.0		
				7,049.0	2,442.0	9,491.0		
		Second	3	3,317.4	1,136.8	4,454.2		
			4	3,522.0	1,136.8	4,658.8		
17	1			6,839.4	2,273.6	9,113.0		
		Third	5	3,131.9	1,114.4	4,246.3		
			6	3,594.7	1,114.4	4,709.1		
				6,726.6	2,228.8	8,955.4		
		Daily average		6,871.7	2,314.8	9,186.5	−37.2	9,149.3
		First	1	2,633.5	863.0	3,496.5		
			2	2,646.8	863.0	3,509.8		
				5,280.3	1,726.0	7,006.3		
		Second	3	2,729.1	867.4	3,596.5		
			4	2,654.0	867.4	3,521.4		
17	3			5,383.1	1,734.8	7,117.9		
		Third	5	2,627.3	831.1	3,458.4		
			6	2,655.2	831.1	3,486.3		
				5,282.5	1,662.2	6,944.7		
		Daily average		5,315.3	1,707.7	7,023.0	+1.9	7,024.9
		First	1	3,162.8	1,380.0	4,542.8		
			2	3,509.3	1,380.0	4,889.3		
				6,672.1	2,760.0	9,432.1		
		Second	3	3,108.8	1,385.0	4,493.8		
			4	3,515.2	1,385.0	4,900.2		
85	4			6,624.0	2,770.0	9,394.0		
		Third	5	3,205.4	1,378.0	4,583.4		
			6	3,470.2	1,378.0	4,848.2		
				6,675.6	2,756.0	9,431.6		
		Daily average		6,657.2	2,762.0	9,419.2	−43.7	9,375.5
		First	1	2,660.3	810.4	3,470.7		
			2	2,824.7	810.4	3,635.1		
				5,485.0	1,620.8	7,105.8		
		Second	3	2,593.7	767.3	3,361.0		
			4	2,785.9	767.3	3,553.2		
17	5			5,379.6	1,534.6	6,914.2		
		Third	5	2,718.7	808.2	3,526.9		
			6	2,882.0	808.2	3,690.2		
				5,600.7	1,616.4	7,217.1		
		Daily average		5,488.4	1,590.6	7,079.0	+0.6	7,079.6

The energy balances show that the animals were on a plane of approximate energy equilibrium.

On the exclusive hay ration (period 1) steer No. 17 showed a slight gain of protein (16.2 gm.) and a slight loss of fat (12.0 gm.), the

energy balance being minus 42 calories. In the exclusive meal ration (period 3) this animal showed a slight loss of protein from the body (47.4 gm.) and a slight gain of fat (43.8 gm.), the energy balance being plus 205 calories. The mixed ration (period 5) caused somewhat greater positive balances of fat and protein. In all cases the energy of the protein has been corrected in the usual manner to nitrogen equilibrium as shown in Table 7.

On the mixed ration of alfalfa hay and corn meal (period 5), which supplied 44 gm. of digestible nitrogen, the animal showed a positive balance of 9.5 gm. of nitrogen, while on the corn-meal ration, supplying 28 gm. of digestible nitrogen, the animal lost from the body 8 gm. of nitrogen. These data show clearly that the maintenance requirement of digestible nitrogen was between 34 and 36 gm. per day. This is somewhat less than the average amount of nitrogen excreted in the urine (39 gm.) in the foregoing fasting experiments. This is undoubtedly due to the protein-sparing effect of the carbohydrates of the rations.

The data of Table 7 show a good agreement between the computed and the observed heat production in all periods—which signifies the absence of any gross errors of heat measurement.

The derivation of the values for observed heat production is given in detail in Table 8. In periods 3, 4, and 5 the daily heat production was very uniform during the 3-day calorimetric periods. These data show no effect of the slight sickness of the animal which was mentioned in the preceding paper. In period 1 the daily heat production was not as uniform as in the other periods and showed a decline from 9,491 calories during the first day to 8,955 calories during the third day.

The data for heat production given in Tables 7 and 8 are uncorrected for difference in time the animals spent in the standing position. In accord with the customary procedure at this institute the heat production has been corrected, in Table 9, to a standard day of 12 hours standing. The factors used as a basis for this correction were those previously obtained in the fasting study with the same animals.

TABLE 9.—*Correction of heat production to a standard day of 12 hours standing*

Period No.	Steer No.	Time spent standing	Difference from 12 hours	Factor [a]	Correction	Observed heat production		Computed heat production	
						Uncorrected	Corrected	Uncorrected	Corrected
		Hours	*Hours*		*Calories*	*Calories*	*Calories*	*Calories*	*Calories*
1	17	7.2	4.8	45.5	+218.4	9,149.3	9,367.7	9,156.0	9,374.4
3	17	8.2	3.8	45.5	+172.9	7,024.9	7,197.8	7,049.9	7,222.8
4	85	7.9	4.1	65.6	+269.0	9,375.5	9,644.5	9,392.7	9,661.7
5	17	8.7	3.3	45.5	+150.2	7,079.6	7,229.8	7,181.2	7,331.4

[a] Determined difference per hour in calories expended in standing and lying.

Before final comparisons were made the data for heat production of each steer were also corrected to a basis of uniform live weight and maintenance requirement. (Table 10.) The heat production of fast was considered as the maintenance requirement of energy, and the maintainance requirement in each experimental period was computed from this base value in proportion to the two-thirds power of the live weight.

TABLE 10.—*Heat production corrected to a basis of uniform live weight and maintenance requirement on a basis of the second day of the fast*

Steer No. and plane of nutrition	Period No.	Average live weight	Fasting katabolism (maintenance requirement)	Correction to be applied to heat production [a]	Heat production			
					Uncorrected for live weight		Corrected for live weight	
					Observed	Computed	Observed	Computed
Steer 17:		*Kilograms*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*
Fast	7	324.1	[b] 5,828	.....	5,828	.....	5,828	.....
Maintenance (hay alone)	1	364.2	6,300	−472	9,368	9,374	8,896	8,902
Maintenance (corn meal alone)	3	327.6	5,870	−42	7,198	7,223	7,156	7,181
Maintenance (mixed ration)	5	338.3	5,997	−169	7,230	7,331	7,061	7,162
Steer 85:								
Fast	12	347.9	[b] 6,752	.....	6,752	.....	6,752	.....
Maintenance (hay alone)	4	332.1	6,546	+206	9,645	9,662	9,851	9,868

[a] Computed in accord with the two-thirds power of the live weight.
[b] Value obtained on the second day of fast.

The results of the fasting experiments indicate that a true fasting status was probably reached on the third day of fast, but these results were not clear-cut, since in some cases the respiratory quotients indicated an oxidation of fat on the second day of fast. For this reason it was deemed desirable to make the corrections for differences in live weight, and the final computation of net energy values of the feeds, on two different bases, using the heat production of the second and also of the third day of fast as the base values. In Table 11 the values for heat production are corrected on the basis of results obtained on the third day of fast (period 7 for steer No. 17 and period 8 for steer No. 85). In Table 10 these corrections are made on the basis of the second day of fast (period 7 for steer No. 17 and period 12 for steer No. 85).

TABLE 11.—*Heat production corrected to a basis of uniform live weight and maintenance requirement on a basis of the third day of the fast*

Steer No. and plane of nutrition	Period No.	Average live weight	Fasting katabolism (maintenance requirement)	Correction to be applied to heat production [a]	Heat production			
					Uncorrected for live weight		Corrected for live weight	
					Observed	Computed	Observed	Computed
Steer 17:		*Kilograms*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*
Fast	7	324.1	[b] 6,011	.....	6,011	.....	6,011	.....
Maintenance (hay alone)	1	364.2	6,498	−487	9,368	9,374	8,881	8,887
Maintenance (corn meal alone)	3	327.6	6,054	−43	7,198	7,223	7,155	7,180
Maintenance (mixed ration)	5	338.3	6,186	−175	7,230	7,331	7,055	7,156
Steer 85:								
Fast	8	335.8	[b] 6,432	.....	6,432	.....	.....	.....
Maintenance (hay alone)	4	332.1	6,385	+47	9,645	9,662	9,692	9,709

[a] Computed in accord with the two-thirds power of the live weight.
[b] Value obtained on the third day of fast.

TABLE 12.—*Heat increments[a] and net energy values, based on the heat production of the third day of fast*

Steer No.	Period No.	Ration		Heat production			Fasting katabolism	Heat increments		Metabolizable energy per kilogram of dry matter	Net energy per kilogram of dry matter	Utilization of metabolizable energy
		Alfalfa hay (dry matter)	Corn meal (dry matter)	Observed	Computed	Average		Total	Per kilogram of dry matter			
		Kilograms	*Kilograms*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Per cent*
17	1	4.827		8,881	8,887	8,884	[b] 6,011	2,873	595	1,888	1,293	68.5
	5	1.499	1.495	7,055	7,156	7,106	[b] 6,011	1,095	366	2,574	2,208	85.8
	3		2.174	7,155	7,180	7,168	[b] 6,011	1,157	532	3,337	2,805	84.1
85	4	4.431		9,692	9,709	9,701	[c] 6,432	3,269	738	1,984	1,246	62.8

[a] Values for corn meal, computed from mixed ration. Heat increment per kilogram of dry matter, 135 calories; metabolizable energy per kilogram of dry matter, 3,262 calories; net energy per kilogram of dry matter, 3,127 calories; utilization of metabolizable energy, 95.9 per cent.
[b] Third day of fast of period 7.
[c] Third day of fast of period 8.

TABLE 13.—*Heat increments[a] and net energy values, based on the heat production of the second day of fast*

Steer No.	Period No.	Ration		Heat production			Fasting katabolism	Heat increment		Metabolizable energy per kilogram of dry matter	Net energy per kilogram of dry matter	Utilization of metabolizable energy
		Alfalfa hay (dry matter)	Corn meal (dry matter)	Observed	Computed	Average		Total	Per kilogram of dry matter			
		Kilograms	*Kilograms*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Per cent*
17	1	4.827		8,896	8,902	8,899	[b] 5,828	3,071	636	1,888	1,252	66.3
	5	1.499	1.495	7,061	7,162	7,111	[b] 5,828	1,283	429	2,574	2,145	83.3
	3		2.174	7,156	7,181	7,169	[b] 5,828	1,341	617	3,337	2,720	81.5
85	4	4.431		9,851	9,868	9,860	[c] 6,752	3,108	701	1,984	1,283	64.7

[a] Values for corn meal, computed from mixed ration. Heat increment per kilogram of dry matter, 221 calories; metabolizable energy per kilogram of dry matter, 3,262 calories; net energy per kilogram of dry matter, 3,041 calories; utilization of metabolizable energy, 93.2 per cent.
[b] Second day of fast of period 7.
[c] Second day of fast of period 12.

The final computations of the heat increments and net energy values of the feeds are given in Tables 12 and 13. In these tables the corrected values for the observed and the computed heat production are averaged, and these average values are compared with the fasting katabolism. In Table 12 the fasting katabolism represents the heat production on the third day of fast, while in Table 13 the heat production on the second day of fast is used as the base value.

The most significant facts brought out in Tables 12 and 13 are that the metabolizable energy value of corn meal as computed from the mixed ration of corn meal and alfalfa hay is practically identical with the metabolizable energy value of the meal as determined directly, and that the heat increment and, therefore, the net energy value of the corn meal as computed from the mixed ration differ considerably from the corresponding directly determined values. In Table 12 the heat increment value of the corn meal fed alone is 532 calories per kilogram of dry matter, while the value computed from the results from the mixed ration was only 135 calories per kilogram. In Table

13 the directly determined value is 617 calories, while the value computed from the results from the mixed ration is only 221 calories per kilogram. The heat increment value of grain as determined, in the usual way, by difference, seems therefore not to give a value representing the energy expense of utilization.

The heat increment values per kilogram of the mixed ration were found to be lower than the heat increment value of either the roughage or the corn meal when fed alone, but were higher than the heat increment value of the corn meal as computed from the mixed ration. These differences apparently represent associative effects of the feeding stuffs in metabolism.

The differences in heat increment directly affect the net energy values. Although the metabolizable energy value of the corn meal, as computed from the mixed ration, was approximately the same as the directly determined value, the net energy value of the meal, as computed from the mixed ration, is considerably greater than the net energy value of the same as directly determined. From this it follows that the net energy value of a feeding stuff may differ in accord with the proportion of the ration which it constitutes.

These results tend to sustain the theory of Forbes [6] that the determination of consistent net energy values implies the presence of proteins, mineral nutrients, and vitamins—in other words, all nutrients except such as serve for energy production—in optimum quantities and proportions.

SUMMARY

The metabolizable energy, heat increment, and net energy values of corn (maize) meal and alfalfa hay were determined separately and in combination.

The metabolizable energy value of corn meal was found to be essentially the same whether fed to steers as the only feedstuff or in combination with alfalfa hay; but the heat increment value of the corn fed by itself was found to be much greater than when fed in the mixed ration. The net energy value of the corn in the mixed ration, therefore, was much greater than when fed alone. From this it follows that the net energy value of a feeding stuff may differ as affected by the proportion of the ration which it constitutes.

This finding is interpreted as tending to sustain the theory of Forbes that the determination of consistent net energy values would require the presence of all nutrients, except such as serve for energy production, in optimum quantities and proportions.

[6] FORBES, E. B. THE PROBLEM OF DETERMINATION OF NET-ENERGY VALUES OF FEEDING STUFFS. Amer. Jour. Physiol. 90 : 348. 1929.

U. S. GOVERNMENT PRINTING OFFICE: 1932